Skeletal muscle modulation and functional recovery after colonic resection

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ABSTRACT

Introduction: Colorectal cancer surgery involves a period of recovery in hospital followed by convalescence at home. Enhanced recovery programs, in particular the use of laparoscopic surgery have reduced length of inpatient stay. Knowledge regarding the impact of surgery upon functional recovery is lacking. Loss of lean muscle mass and strength may compromise function. We conducted two studies (1) To assess skeletal muscle modulation and functional recovery after potentially curative colon cancer surgery, comparing traditional open surgery (OS) with laparoscopic (LS) techniques and (2) To review return to work (RTW) 1 year post colorectal cancer surgery.

Methods: (1) A prospective longitudinal observational study was conducted at a single UK institution (April 2013 and December 2014). Participants undergoing OS and LS for colon cancer were recruited preoperatively and assessed over 6 months. The study was powered to changes in hand grip strength (minimum sample size of 24 patients in each group), and included analysis of serological inflammatory markers (granulocyte lymphocyte ratio (GLR)); muscle architecture (pennation angle (PA), muscle thickness (MT) and fascicle length (FL)), muscle protein synthesis rate and assessment of function (numerical pain score, dukes activity status index (DASI)) and health status (EQ5d5L).

(2) A retrospective cohort questionnaire study was conducted. A specific questionnaire was created and dispatched to 204 patients who had undergone surgery with curative intent for colorectal cancer within a single teaching hospital in 2011-2012.

Results: (1) Fifty-three patients (OS n=27; LS n=26) were recruited with no statistical differences between groups (age, sex, body mass index, tumour stage, blood loss). LS associated with longer mean operating time (182.5mins v 142.1mins, $p<0.05$), fewer complications ($p<0.05$) and shorter length of stay (3 days v 5 days, $p<0.05$).

Hand grip decreased post surgery (maximum decrease day 3 (OS 24% v LS 15%, $p<0.05$), with OS data significantly lower at 2, 4 and 6 weeks ($p<0.05$)). GLR peaked on day 1 post surgery with no difference between groups at any time point. Muscle architecture assessment noted OS associated with decreased MT (8% v 1%, $p<0.05$) and PA (6% v 1%, $p<0.05$) at 4 and 6 weeks post surgery. Muscle protein synthesis rate for OS was 1.02±0.02%/day. OS pain scores were significantly higher at 2, 4 and 6 weeks ($p<0.05$). EQ5d5L and DASI scores were significantly lower for OS at 2, 4, 6 weeks and 6 months ($p<0.05$).

(2) Response rate was 75% (OS=82%, LS=51%). LS reported earlier 'return to full fitness' (1-3 months) than OS (>6 months; $p<0.05$). Recovery from LS was 'better than expected' compared to OS 'worse than expected' ($p<0.05$). Forty-nine patients were employed preoperatively and 61% (n=30) returned to work. RTW was more frequent after LS ($p<0.05$). Length of time to RTW was significantly less after LS [44 (6-84) days] than OS [71 (14-252) days] ($p<0.05$).

Conclusions: OS was associated with increased loss of strength, muscle mass and reduced MPS in the first six weeks after surgery, together with poorer functional recovery including RTW. One-third of patients failed to RTW 1 year post colorectal cancer surgery. We must invest more in managing expectations and provide better post discharge support to improve long term functionality.

PUBLICATIONS & PRESENTATIONS

Publications

Bhalla A, Williams JP, Hurst NG, Speake WJ, Tierney GM, Tou S, Lund JN. One-third of patients fail to return to work 1 year after surgery for colorectal cancer. Tech Coloproctol. 2014 Dec;18(12):1153-9. doi: 10.1007/s10151-014-1232-y. Epub 2014 Nov 8. PMID: 25380740

Presentations

The Association of Coloprotology of Great Britain & Ireland

Tripartite meeting 2014

LTP015 Laparoscopic colorectal cancer surgery leads to a quicker full recovery after discharge, but over one-third of working patients never return to work. Colorectal Disease. Volume 16, Issue s2, July 2014, Pages: 41–68, Article first published online : 22 AUG 2014, DOI: 10.1111/codi.12643

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O43 Functional recovery takes 4 times as long after open resection for colon cancer compared to laparoscopic resection.

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I reserve my last thank you to the patients. Having spent time with each individual I have only admiration for their strength, bravery and honesty. Being diagnosed with cancer and subsequently undergoing treatment is life changing, but to go through that and still volunteer your time to undergo additional, occasionally painful tests is humbling. Without them none of this would be achievable. They were inspirational, thank you.

DECLARATION

I hereby declare that the work presented in this thesis is my own.

Ashish Bhalla

CONTENTS

ABBREVIATIONS

APE	Atomic percentage enrichment
APR	Abdomino-perineal resection
BI	Bioelectrical impedance
BMI	Body mass index
CRP	C-reactive protein
CT	Computed tomography
D_2O	Deuterium oxide
DASI	Duke's activity status index
DXA	Duel X-ray absorptiometry
EDTA	Ethylenediaminetetraacetic acid
ERAS	Enhanced recovery after surgery
EQ5d5l	EuroQuol 5-dimension questionnaire (English language edition)
FL	Fascicle length
GLR	Granulocyte lymphocyte ratio
IL-6	Interleukin-6
MPB	Muscle protein breakdown
MPS	Muscle protein synthesis
MRI	Magnetic resonance imaging

MT	Muscle thickness
NICE	National institute of health & care excellence
NRS	Numerical rating score
PA	Pennation angle
POM	Point of measurement
RTW	Return to work
SD	Standard deviation
SEM	Standard error of the mean
SMACC	Skeletal muscle modulation after colon cancer resection
SSR	Surgical stress response
TME	Total mesorectal excision
VAS	Visual analogue scale
VL	*Vastus lateralis*

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Chapter 1

Introduction

The definitive cure for patients diagnosed with colorectal cancer is surgery. Surgery disrupts skeletal muscle architecture, impinging upon its ability to function. In the majority of cases this disruption is temporary, and normal activities resume once the body has healed sufficiently. In patients undergoing major surgery this initial disruption is followed by a degree of modulation caused by complex inflammatory, metabolic and endocrine responses commonly referred to as the surgical stress response (SSR). Skeletal muscle modulation is associated with loss of lean muscle mass thereby delaying time to full functional recovery.

This thesis explores skeletal muscle modulation and functional recovery in patients undergoing elective surgery for colon cancer. Chapter 1 highlights the surgical options available to patients, our current understanding of skeletal muscle modulation, and reviews interventions aimed at reducing muscle mass losses to improve recovery.

1.1 COLORECTAL CANCER

1.1.1 Epidemiology

Colorectal cancer is the third commonest cancer in the United Kingdom, with approximately 100 new cases diagnosed each day (NICE 2011). The term includes cancerous growths arising from the colon, rectum and appendix.

The development of colorectal cancer strongly correlates with age: 83% of cases arise in people over the age of 60 (NICR 2008; WCIS 2008; ONS 2008a). Until

the age of 50, the incidence in men and women is similar, however in later-life colorectal cancer in males becomes predominant.

The aetiology of colorectal cancer is multifactorial. Risk factor modification initiatives led by Department of Health have been in-effective, although screening potential may have a positive effect upon survival (table 1.1).

Risk factors	Risk reduction initiatives
Age	National screening programme (DoH)
Polyps	UK surveillance guidelines (NICE)
Inherited disorders	UK surveillance guidelines (NICE)
Inflammatory Bowel disease	UK surveillance guidelines (NICE)
Obesity	Change4life (DoH)
Diet	5-a-day (DoH)
Smoking	New Leaf (DoH)
Alcohol	Drink Aware! (DoH)

Table 1.1 Colorectal cancer risk factors & risk reduction initiatives

Department of Health (DoH); National institute of health and care excellence (NICE)

1.1.2 Clinical presentation

Patients with colorectal cancer either present acutely as an emergency or urgently via a referral to a specialist (elective referral). Individual presentations vary with a wide range of common symptoms and clinical signs (table 1.2).

Symptoms	Clinical signs
PR bleeding	Anaemia
Altered bowel habit	Abdominal mass
Pain	Abdominal tenderness
Bloating	Bowel obstruction
Weight loss	Cachexia
Reduced appetite	Abdominal distension
Tiredness	Peritonitis

Table 1.2 Common symptoms & clinical signs for colorectal cancer.

The left side of the colon is affected by cancer more often than the right (figure 1.1), with tumours of the sigmoid colon, recto-sigmoid junction and the rectum accounting for over half of all cases within the United Kingdom (NICR 2008; WCIS 2008; ONS 2008a).

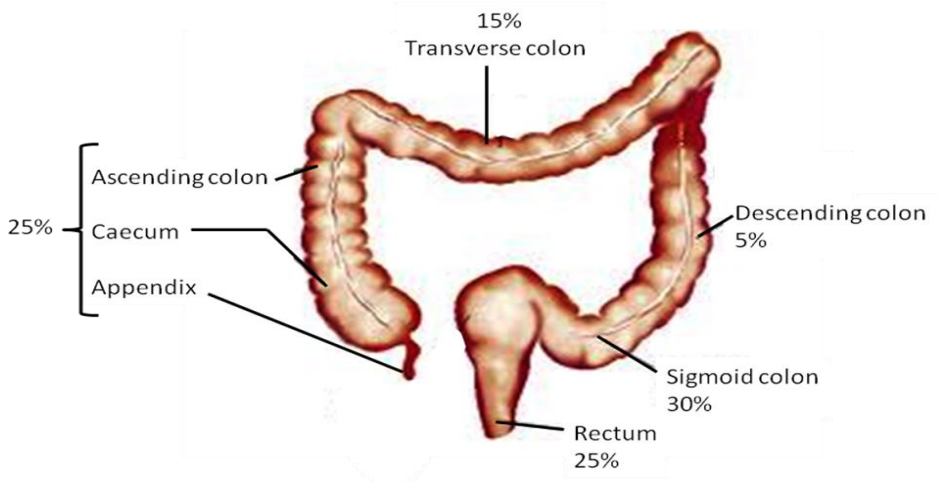


Figure 1.1 Incidence of cancer within the colon and rectum.

Anatomy of the colon and rectum highlighting the incidence of cancer at various sites as a percentage of all colorectal cases.

1.1.3 Diagnosis & management

Once colorectal cancer is suspected, the majority of patients follow a structured clinical pathway in order to confirm the diagnosis and commence treatment (figure 1.2).

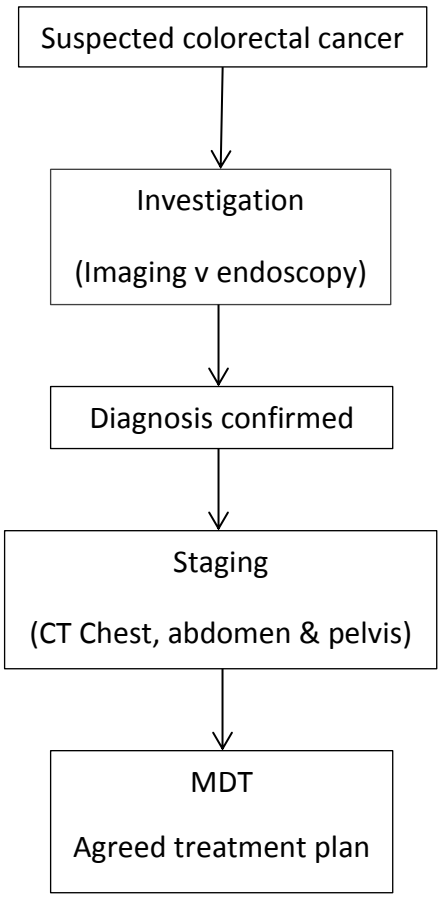


Figure 1.2 Pathway for colorectal cancer management

The treatment pathway undertaken by patients suspected of suffering from colorectal cancer. Surgery is offered after review of histology and imaging at the multidisciplinary team meeting. Computer tomography (CT); Multidisciplinary team meeting (MDT)

The aim of investigation is to achieve adequate examination of the entire colon and rectum. The pre-requisite for an effective diagnostic intervention is to be highly sensitive for the detection of cancers or adenomas with significant potential for malignant transformation (Scholefield 2000). Several investigation modalities are available to a clinician, including endoscopy and radiological imaging (ie. barium enema or computer tomographic colography). Both allow visualisation of the bowel, however endoscopy has an advantage of enabling biopsies to be taken simultaneously; providing samples for histological confirmation of malignancy. Histologically, the overwhelming majority of colorectal malignant neoplasms are adenocarcinomas (approximately 95%) whilst the remaining cancer-cell types include lymphomas, squamous cell carcinomas and carcinoid tumours (Fearon and Vogelstein 1990). The treatment options are tailored to each individual cancer-cell type. This thesis focuses on the treatment of patients suffering from adenocarcinoma only.

After a diagnosis of colorectal adenocarcinoma has been confirmed, knowledge of local invasiveness and distant spread is essential in planning treatment. For colon cancer, this is usually achieved with the aid of a contrast enhanced computed tomography scan (CT) of the chest, abdomen and pelvis. The TNM5 classification (table 1.3) is the system currently used for staging colorectal cancer in the UK. Once a patients' disease state has been incorporated into the TNM scoring system, the disease can be accurately staged in order to create an effective evidence based treatment plan (table 1.4).

Survival is stage dependent (table 1.5) and over the last 25 years there have been significant improvements in survival for both colonic and rectal cancers (Coleman, Rachet et al. 2004; CRUK 2009). These improvements are as a direct

result of earlier diagnosis and improved surgical and oncological treatment options. Earlier tumour diagnosis was achieved through the national bowl cancer screening programme, sanctioned by the Department of Health in 2009 after a successful pilot programme in 2006. The screening programme offers all men and women aged between 60 and 69 an invitation letter and information leaflet. If interested each participant receives a faecal occult blood test kit and step-by-step instructions for completing the test at home and sending samples to the screening laboratory. Participants with a negative test are reassured and re-screened every two years, whilst participants with a positive test are referred for a colonoscopy (NICE 2011).

Primary tumour (T)	Pathological characteristics
T1	Tumour is confined to the submucosa
T2	Tumour has grown into (but not through) the muscularis propria
T3	Tumour has grown into (but not through) the serosa
T4a	Penetrated through the serosa and peritoneal surface and extending directly into other nearby structures
T4b	Penetrated through the serosa and peritoneal surface with perforation of the bowel
Lymph nodes (N)	
N0	No lymph nodes contain tumour cells
N1	Tumour cells in up to 3 regional lymph nodes
N2a	Tumour cells in 4-6 regional lymph nodes
N2b	Tumour cells in 7 or more regional lymph nodes
Metastases (M)	
M0	No metastasis to distant organs
M1a	Metastasis to distant organ
M1b	Multiple organs with metastatic deposits

Table 1.3 Tumour Node Metastasis (TNM) classification (5th edition).

Stage	TNM Classification
I	T1 N0 M0, T2 N0 M0
IIA	T3 N0 M0
IIB	T4a N0 M0
IIC	T4b N0 M0
IIIA	T1-T2 N1 M0, T1 N2a M0
IIIB	T3-T4a N1 M0, T2-T3 N2a M0, T1-T2 N2b M0
IIIC	T4a N2a M0, T3-T4a N2b M0, T4b N1-2 M0
IVA	Any T Any N M1a
IVB	Any T Any N M1b

Table 1.4 Numerical staging systems for colorectal cancer

Colorectal cancer stages with corresponding TNM scores.

TNM Stage	Frequency at diagnosis	5 year survival
I	11%	83%
II	35%	64%
III	26%	38%
IV	28%	3%

Table 1.5 Frequency & 5 year survival rates for colorectal cancer

Modified with permission from the National Institute of Health and Care Excellence

Each case is discussed within a local colorectal cancer multidisciplinary team meeting (MDT) and a consensus on best plan for treatment is reached. The treatment for colonic and rectal cancers differs depending upon the exact stage of the disease and each patient's co-morbidity. The National Institute for Health and Care Excellence (NICE) has issued treatment guidelines for regional MDT's for the treatment of colonic (figure 1.3) and rectal cancers (figure 1.4).

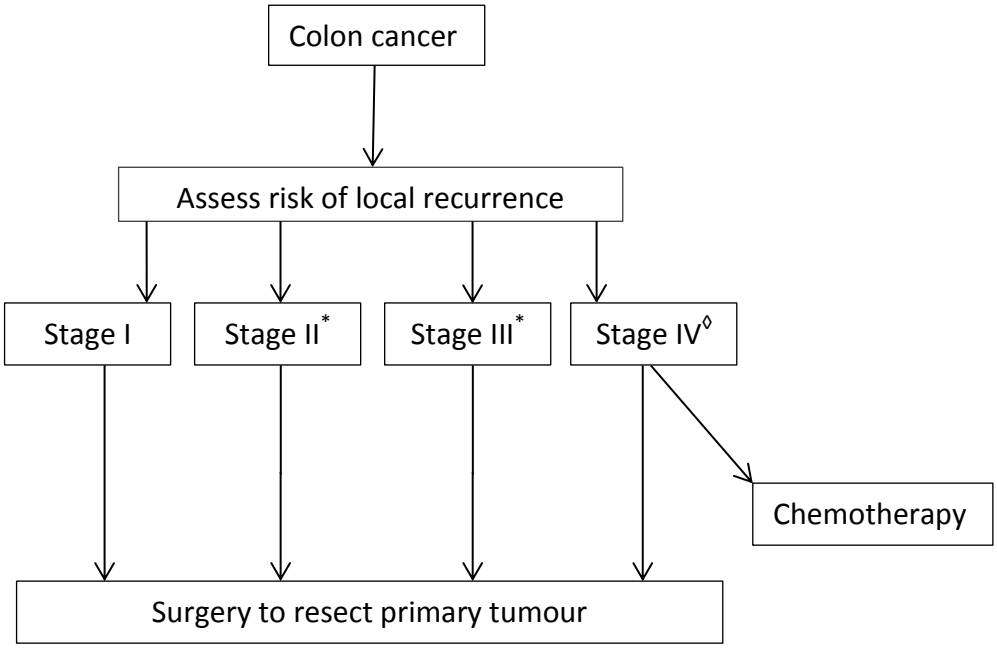


Figure 1.3 Treatment algorithms for colon cancer

** Cases need to be assessed on an individual basis to ascertain whether patients will benefit from postoperative chemotherapy after resection of the primary tumour depending upon postoperative re-staging. ◊ Cases need to be assessed on an individual basis in order to highlight patients which may benefit from surgical resection of their primary tumour followed by potentially curable surgery for metastatic disease. The majority of patients have inoperable primary tumours or unresectable metastatic disease and are offered palliative chemotherapy only. Modified with permission from the National Institute of Health and Care Excellence.*

In patients diagnosed with rectal cancer, local recurrence is a particular problem. Accurate pre-treatment staging identifies characteristics which predict local recurrence and helps determine appropriate treatment strategies to minimise recurrence. The most important characteristic in predicting local recurrence is infiltration of the proposed circumferential resection margin. This is highlighted with accurate preoperative imaging, usually a pelvic magnetic resonance imaging scan (MRI). Patients with inoperable rectal cancer are offered palliative chemo-radiotherapy alone.

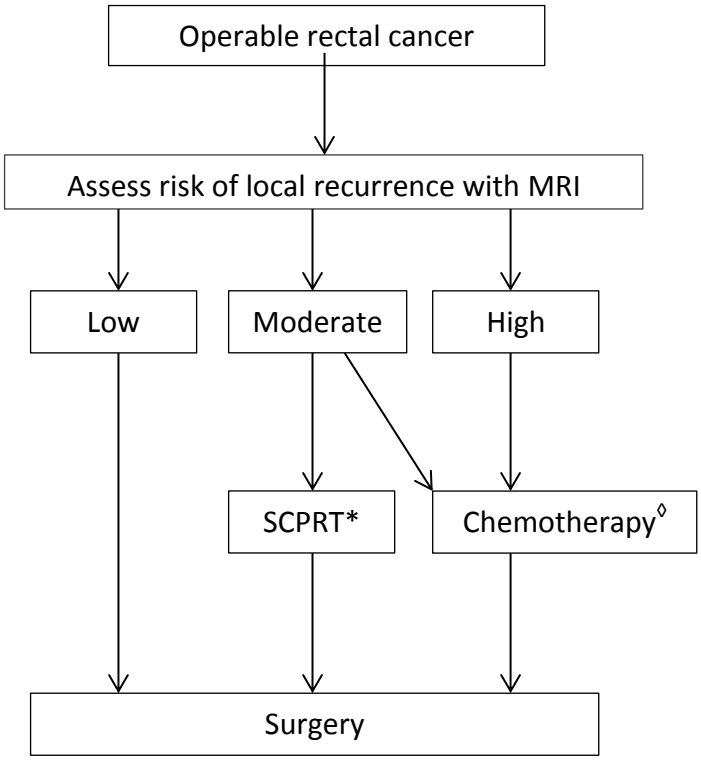


Figure 1.4 Treatment algorithms for operable rectal cancer

** Patients at significant risk of local recurrence may be offered short course preoperative radiotherapy (SCPRT) prior to surgery. ◊ Patients at high risk of local recurrence may be offered chemoradiotherapy prior to delayed surgery allowing time for the tumour to respond to neoadjuvant treatment. Magnetic resonance imaging (MRI). Modified with permission from the National Institute of Health and Care Excellence*

1.1.4 Open colorectal surgery

Surgery is the only curative treatment for localized colorectal cancer (stage I-III) and potentially provides a curative option for patients with resectable metastatic disease affecting the lung or liver (stage IV).

The general principle for any colorectal resection is to remove the primary tumour with adequate disease-free margins including areas of potential lymphatic drainage. Several surgical options exist, with the exact choice of operation dependent upon the anatomical position of the primary tumour (figures 1.5 & 1.6). All open operations require access to the abdominal cavity through a single incision large enough to allow the surgeon and assistant to operate freely with both hands.

Segmental colectomy, with division of appropriate named artery supplying that segment, is performed for colonic tumours. As lymphatics run with named arteries lymph nodes required for staging and regional control of the tumour are removed *en bloc* (figure 1.5). An anterior resection or abdominoperineal excision is performed for lesions of the rectum (figure 1.6A).

Rectal mobilization requires incorporation of a technique named 'total mesorectal excision' (TME). TME included excision of the rectum and its mesentry (surrounding tissue containing the rectal blood supply and lymphatic drainage) (together with neoadjuvant chemoradiotherapy) significantly improves local recurrence rates of rectal cancer (Nelson, Petrelli et al. 2001).

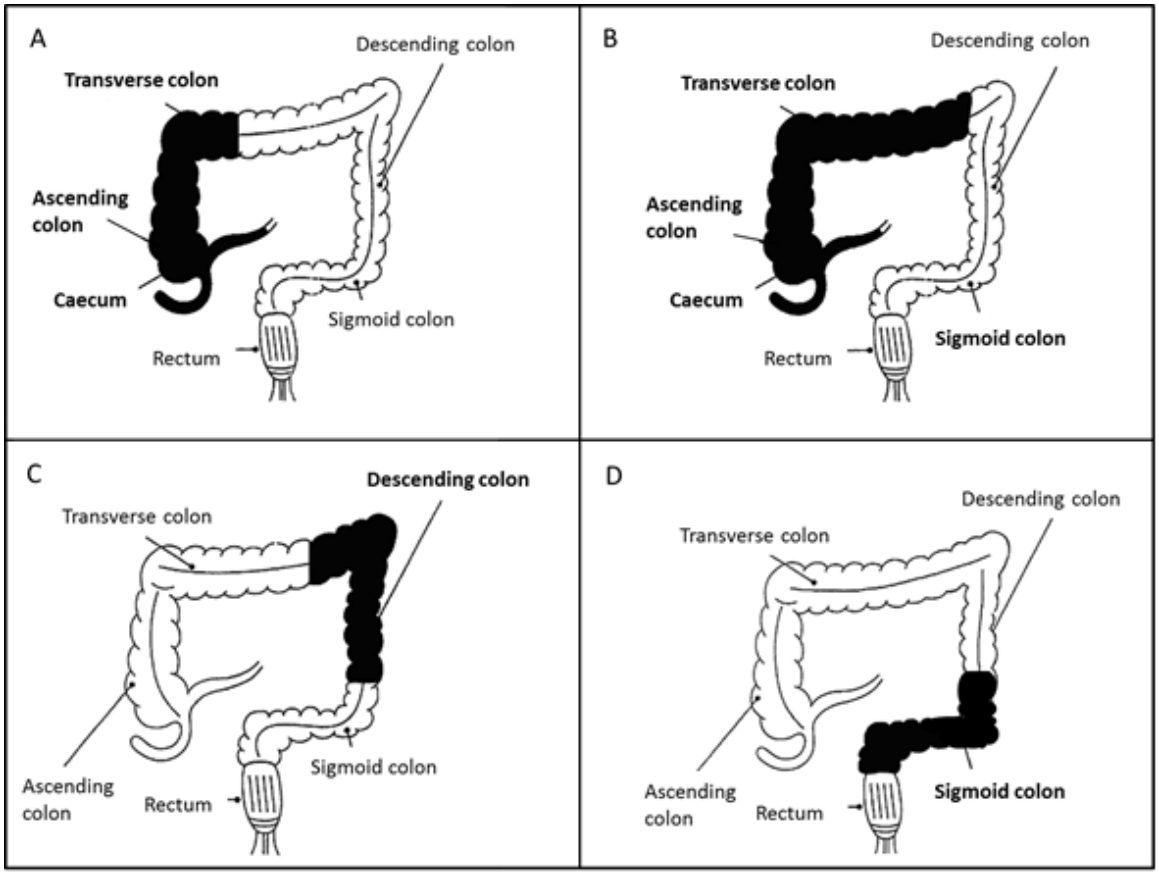


Figure 1.5 Common colonic operations

Excised areas in bold. A- right hemi-colectomy; B- extended right hemi-colectomy; C- left hemi-colectomy; D- sigmoid colectomy. Cancers found within the descending and sigmoid colon undergo a left hemicolectomy as the inferior mesenteric artery is ligated at its origin in order to excise associated lymph nodes to allow for accurate staging of the disease. Sigmoid colectomy is not performed for sigmoid colonic adenocarcinoma. Modified with permission from the Royal United Hospital Bath NHS Trust

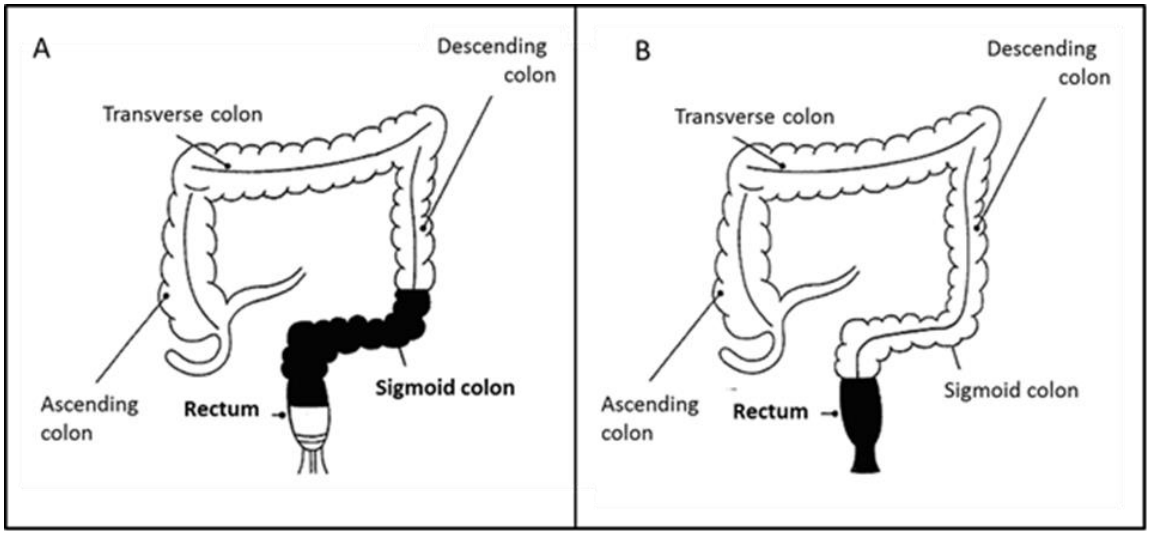


Figure 1.6 Common operations for rectal cancer

Excised areas in bold. A- anterior resection; B- abdomino-perineal resection. Modified with permission from the Royal United Hospital Bath NHS Trust

It follows intuitively that adequate *en bloc* clearance of the rectum and its mesentery minimizes the possibility of local recurrence without neoadjuvant or adjuvant treatment. TME was first described in 1982 by Heald and involves dissection within the avascular plane created by an envelope that separates the mesorectum from the surrounding structures (Heald, Husband et al. 1982). This includes the peritoneal reflection and Denonvilliers fascia anteriorly, whilst preserving the inferior hypogastric plexus posteriorly and laterally. The introduction of TME has resulted in an impressive reduction of local recurrence rates and improved overall survival in patients without systemic disease (Maurer, Renzulli et al. 2011). It must also be noted that TME is associated with a significantly longer operation time than mesorectal excision alone (Fujita, Akasu et al. 2012).

Distal resection margins vary depending upon the exact site of the lesion. A 1-2 cm margin distal to the lesion must be achieved. For distal rectal cancers (less than 5 cm from the anal verge) the minimally accepted distal margin is 1 cm. Distal intra-mural spread beyond 1 cm is rare and associated with aggressive tumour behaviour or advanced tumour stage.

An abdomino-perineal resection (APR) is performed in patients with very low rectal cancers in whom an adequate distal resection margin will result in loss of the anal sphincter or in patients with pre-existing sphincter dysfunction or pelvic fixation (figure 1.6B). The left colon and rectum are again mobilised, followed by transection of the colon proximally to create an end-colostomy. The anus is closed with a purse-string suture. A circumferential perianal elliptical incision is made and extended caudally to reach the level of the *levator ani* muscles. The surgeon retrieves the large specimen placed within the pelvis from below.

Patients undergoing surgery for colorectal cancer are often elderly and may be further debilitated by chronic diseases. The abdominal wound alone is a major factor contributing to morbidity for these patients.

The high concentration of bacteria within stool contributes to high rates of wound infections, dehiscence and eviscerations. In addition, pain from the abdominal wound often compromises pulmonary function and limits mobility in the immediate postoperative period and full recovery after discharge (Grosso, Biondi et al. 2012). As a result, minimally invasive techniques (laparoscopic surgery), which limit the size of the abdominal wound potentially offer distinct advantages (figure 1.7).

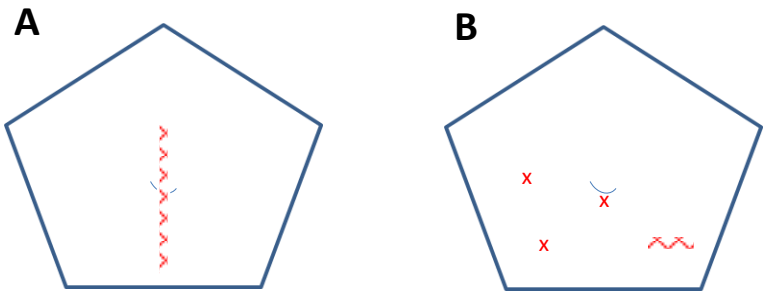


Figure 1.7 Incisions for open and laparoscopic anterior resection

Representation of an abdomen and the incisions required for surgery. A- Open surgery; B- Laparoscopic surgery. Hatched areas represent incisions and crosses represent laparoscopic port sites (0.5-1cm). Open procedures require a relatively large incision for surgery whilst laparoscopic surgery requires a 5-8 cm incision for specimen retrieval only.

1.1.5 Laparoscopic colorectal surgery

Laparoscopy is the inspection of the abdomen or pelvis through small incisions with the aid of a camera. It is used to diagnose conditions, and perform surgery with the aid of specialist instruments.

The advent of laparoscopy has revolutionized the surgical approach to colonic and rectal resections for cancer. The same oncological principles are respected as for open surgery, namely, excision of the primary tumour with adequate disease free margins and incorporation of potential sites of lymphatic drainage.

The use of laparoscopy is intrinsically linked to advances in technology (table 6). Over time, problems that plagued early enthusiasts diminished; specifically iatrogenic injuries (both thermal and instrument related) and the absence of scientific knowledge regarding the dangers of lengthy periods of increased intra-abdominal pressure.

The first laparoscopic colorectal surgery was performed by De Kok, a gynaecologist in 1977. He mobilised and removed the appendix, retrieving it through a small laparotomy(de Kok 1977). This was followed by Semm who performed the first completely laparoscopic appendectomy in 1983. His technique consisted of an extracorporeal ligation of the mesoappendix with endoscopic ligation of the appendix with a pre-tied loop. The appendix was transected across its base with electrocautery (Semm 1983).

Year	Surgeon	Achievement
1901	Ott	Introduced a speculum through the posterior vaginal fornix (Ventroscopy)
1901	Kellig	Inserted a cystoscope intra-abdominally in canines (Celioscopy)
1910	Jacobeus	Conducted celioscopy in humans
1911	Bernheim	Pioneered organoscopy by publishing a review of several cases in humans
1923	Kellig	Reported 22 year experience of laparoscopy
1929	Kalk	Introduced idea of pneumo-peritoneum and created a lens light system
1938	Veres	Introduced the retractable needle, minimising bowel perforation
1952	Forrest	Utilized a quartz rod to transmit a light beam along a telescope
1959	Semm	Introduced first camera into the abdomen
1966	Semm	Created first insufflation device
1970	Semm	Perfected the EndoLoop applicator & diathermy
1978	Hassan	Developed a safe technique for insufflation

Table 1.6 Evolution of laparoscopic surgery (1901-1978)

Application of laparoscopic techniques to colorectal operations was initially limited by the lack of appropriate instruments. Early laparoscopic resections of the colon were laparoscopic-assisted resections, where mini-laparotomies were utilized for ligation of mesenteric vessels, extracorporeal anastomoses, and specimen retrieval. The first completely laparoscopic colonic resection using this technique was a right hemicolectomy, performed by Moises Jacobs in Miami, Florida, in June 1990 (Jacobs, Verdeja et al. 1991).

Later that year, Dennis Fowler performed the first laparoscopic sigmoid resection and one month later Patrick Leahy was able to resect a proximal rectal cancer and construct the anastomosis with a specially constructed stapling device.

1.1.6 Laparoscopic versus open colorectal surgery: Review of the evidence

Over time there have been numerous studies focusing upon the role of laparoscopic surgery as an alternative to open surgery for colorectal cancer. In 2005 a Cochrane meta-analysis reviewing short-term outcomes after laparoscopic colorectal surgery included 25 randomised controlled trials and reported that a laparoscopic approach was associated with increased operating time but less intraoperative blood loss compared with open surgery. Furthermore, postoperative pain was significantly less, duration of postoperative ileus shorter, pulmonary function improved, morbidity decreased, and quality of life in the first month better, after laparoscopy compared with open surgery. The authors concluded that long-term outcomes of laparoscopic and open procedures needed to be analysed but if they also showed equivalent results, the laparoscopic approach should be preferred in colorectal cancer surgery (Schwenk and Kehlet 2004).

A second Cochrane meta-analysis published in 2008, reviewed long-term outcomes after laparoscopic colorectal surgery compared with open techniques. Twelve randomised controlled trials were included and factors including number of harvested lymph nodes and positive resection margins were analysed.

Laparoscopic surgery was found to be associated with a significantly lower average number of lymph nodes harvested than open surgery (p=0.003), although this did not affect 5 year survival rates (Kuhry, Schwenk et al. 2008). Results for positive resection margins were the same for either technique. Overall, the meta-analysis concluded, long-term outcome after laparoscopic surgery for colon cancer was no different to outcomes after open surgery; however for rectal cancers alone, the number of available studies and recruited patients was too low to draw any reliable conclusions.

A third meta-analysis published in 2012, compared laparoscopic and open resections for rectal cancer alone. It incorporated data from 9 randomised controlled trials and confirmed laparoscopic surgery benefited patients through a shorter hospital stay, earlier return to bowel function and reduced intra-operative blood loss. The paper went further highlighting several longer-term benefits including, fewer episodes of postoperative blood transfusion, adhesional intestinal obstruction and a lower rate of morbidity. Therefore the intraoperative and long-term oncological outcome of laparoscopic rectal surgery was comparable to that of open surgery (Trastulli, Cirocchi et al. 2012).

The most recent meta-analysis, published in 2013 reviewed both the long-term and short-term outcomes of laparoscopic and open surgery for colon cancer alone. It too re-iterated all the benefits of laparoscopic surgery listed above but surprisingly, the analysis noted no significant overall difference between laparoscopic and open surgery, stating that the operating costs were higher and the hospitalization costs were lower for laparoscopic surgery compared with open surgery (Ohtani, Tamamori et al. 2012).

The use of laparoscopic surgery is now widespread amongst colorectal surgeons, with NICE guidelines for the United Kingdom recommending laparoscopic (and laparoscopic-assisted) resection as an alternative to open resection for any individuals with colorectal cancer in whom both laparoscopic and open surgery are considered suitable (NICE 2006).

1.2 SKELETAL MUSCLE MODULATION

1.2.1 Background

We all require skeletal muscle for every activity of daily living, from the essential tasks of eating and breathing to more complex coordinated movements required for interaction with the world around us. In addition, skeletal muscle is vital for maintaining metabolic health, being essential in lipid and glucose metabolism, and central in control of insulin sensitivity.

Skeletal muscle is formed by multiple bundles of muscle fibres. Each individual muscle fibre (fascicule) is created by the fusion of several undifferentiated immature cells (myoblasts) into a long, cylindrical, multi-nucleated cell. Every fibre is packed with protein, principally myosin and actin, which are uniquely arranged into repeating units called sarcomeres. Complex interactions between myosin and actin are responsible for producing a contraction and several bundles contracting together produces co-ordinated movement.

1.2.2 Assessment

Direct measurement of muscle protein synthesis (MPS) and muscle protein breakdown (MPB) provides relevant information regarding the metabolic state of muscle and should be included as an outcome variable when examining anabolic/catabolic conditions.

1.2.2.1 Muscle protein synthesis

Labelled amino-acid studies

Traditionally protein synthesis rates are measured by administering an amino-acid as a tracer labelled with an isotope of carbon, hydrogen or nitrogen (either radioactive or stable). The rate at which labelled amino-acid is incorporated into muscle protein, as a function of the amount of labelled amino-acid in the precursor pool at the site of translation, directly reflects the rate of protein synthesis.

Originally MPS rates were measured using radioactively labelled amino-acids. These studies made a massive contribution to our understanding of muscle protein metabolism. The risks from radiation exposure has limited their use in humans, however radioactive isotopes have largely been replaced by stable isotopes of the same element (table 1.7).

Element	Normal	Stable	Radioactive
Hydrogen (H)	^{1}H	^{2}H	^{3}H
Carbon (C)	^{12}C	^{14}C	^{13}C
Nitrogen (N)	^{14}N	-	^{15}N
Oxygen (O)	^{16}O	-	^{17}O ^{18}O

Table 1.7 Stable and radioactive isotopes used in amino-acid studies

In most respects experiments with stable isotopes are the same as those with radioactive isotopes, except the term 'isotopic enrichment' replaces the term 'specific radioactivity'. Enrichment is the number of labelled molecules expressed as a proportion of the total number of molecules of the compound, and is measured with a mass spectrometer (Garlick and Cersosimo 1997).

Direct incorporation methods using tracers such as [13C]leucine or [13C]-, [15N]-, or [2H]phenylalnine have been used to measure tissue specific protein synthesis and are generally accepted for providing reliable measurements. The method requires administration of a known amount of labelled amino-acid (tracer) and a known amount of unlabelled amino acid (tracee). The tracer and tracee mix within the body's endogenous pool of amino-acids and become incorporated into proteins over time. The tracer and tracee are either administered as a primed-constant infusion (a priming bolus of tracer and tracee is administered and then continuously provided at a lower concentration to maintain enrichment), or as a flooding dose (a large bolus of tracer and tracee is provided over seconds to minutes) (Gasier, Fluckey et al. 2010).

MPS is determined by measuring the enrichment (tracer/tracee) of muscle protein against the enrichment of the precursor (i.e., precursor:product labelling ratios) by taking a muscle biopsy and measuring the relative amounts of each with a mass spectrometer (Gasier, Fluckey et al. 2010).

Two central concerns exist with the precursor-product method, namely; identification and subsequent measurement of the true precursor, and the difficulty in conducting these experiments within living environments over an extended period of time. With regards to isolating and measuring the precursor (a small pool with a rapid turnover), investigators have either used surrogates (i.e., plasma and/or protein-free compartments), or implemented the flooding dose approach.

Researchers directly comparing the labelling ratios of surrogates to the tRNA-bound amino-acids (e.g., leucyl or phenylalanyl) have reported inconsistencies as to which surrogate most accurately reflects the precursor (Watt, Lindsay et al. 1991; Baumann, Stirewalt et al. 1994; Caso, Ford et al. 2002; Chow, Albright et al. 2006). In addition, although the flooding dose may minimize labelling gradients by saturating the uptake of the amino acid by a tissue, questions exist as to whether MPS is actually stimulated by the large doses of labelled amino-acids used as tracers, especially in a fasting subject (Garlick, McNurlan et al. 1980; Jahoor, Zhang et al. 1992; Smith, Reynolds et al. 1998). In a subject who is fed as part of an experimental protocol the effect of feeding itself may actually stimulate MPS and any ingested protein may potentially alter the precursor:tracer ratio.

The difficulty in conducting tracer experiments within living environments is that the experiments require placement of cannulas within large veins (e.g. the femoral vein) to infuse the tracers and extract blood samples in order to ensure steady state enrichments are maintained over the course of the study.

Deuterium oxide

An alternative, but less familiar approach which bypasses many of the potential limitations of labelled amino-acid studies is the use of deuterium oxide (heavy water or $^{2}H_2O$ or D_2O). D_2O was first discovered in 1932 by Harold Urey, a discovery that subsequently earned him a Nobel Prize. D_2O contains a large amount of the hydrogen isotope deuterium (^{2}H) in comparison with the hydrogen-1 isotope (protium) present in normal water (figure 1.8).

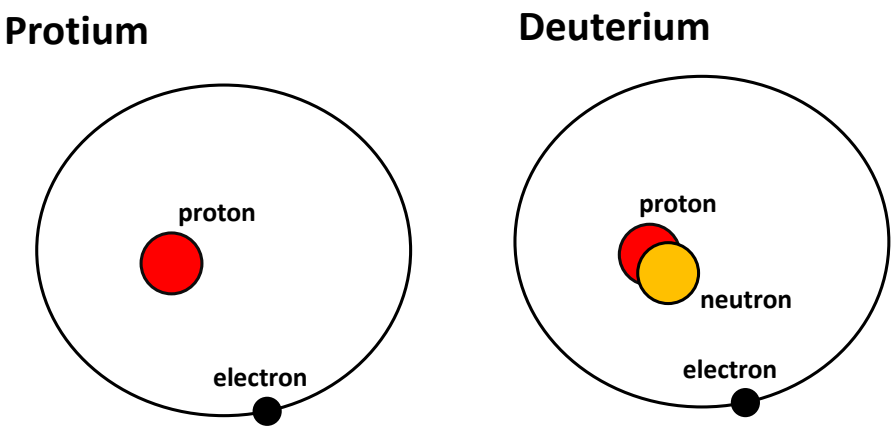


Figure 1.8 Atomic structures of the hydrogen isotopes.

The hydrogen atoms within D_2O contain an additional neutron, causing each deuterium isotope to be almost twice as heavy as protium (figure 1.9). The actual weight of the subsequent D_2O molecule is not substantially affected, due to the fact that over 80% of the molecular weight of water resides within the oxygen atom (atomic number 16).

Although physically similar to normal water, being clear and odourless, D_2O has higher melting and boiling points and is more viscous, with a density about 11% greater than H_2O. Within naturally occurring water there are only approximately 156 deuterium atoms per million protium atoms, whilst within D_2O deuterium atoms account for 99% of all the hydrogen atoms present. Until recently the main uses of D_2O have been within industry, where its use as a moderator within pressurized nuclear reactors has revolutionised nuclear power production.

Over the last twenty years attention has focused upon the possibility of using D_2O within clinical practice. Slight variations in the chemical and physical properties of D_2O in comparison to normal water are directly due to isotopy and its ability to form extremely strong bonds. These variations affect the way in which D_2O behaves with living systems when compared with normal water. The effects of D_2O within living systems may be grouped as either *solvent isotope effects* (based upon properties of the molecule as a whole and its effects upon the structure of water and other macro-molecules) or the *deuterium isotope effect* (the ability of D_2O to replace protium with deuterium within biological molecules because the carbon-deuterium bond is several times stronger than the carbon-protium bond and thus more resistant to enzymatic and chemical cleavage).

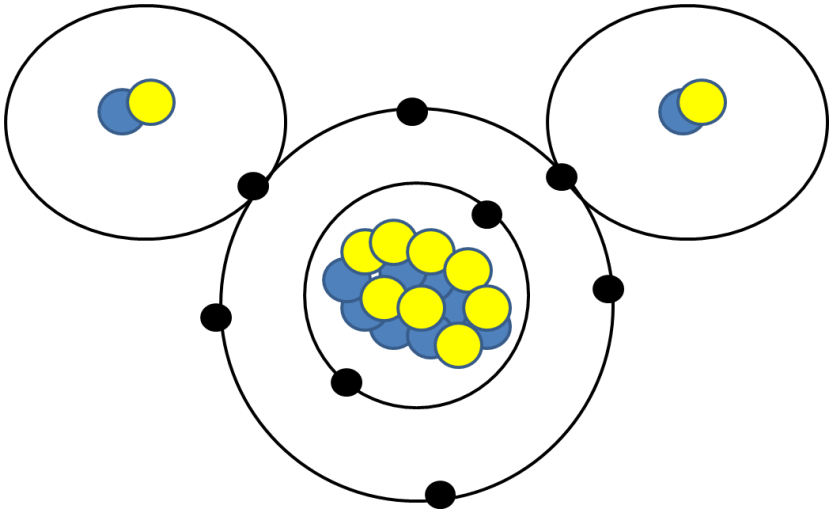


Figure 1.9 The deuterium oxide molecule.

Two deuterium atoms bond with an oxygen atom. Protons (yellow); Neutrons (blue); Electrons (black).

The use of D_2O as a biological tracer was first described by Hans Ussing in 1941 (Ussing 1941). The major advantages of D_2O compared to other biological tracers are its non-radioactivity, (therefore less harmful to individuals being investigated), and more importantly its ability to be rapidly distributed to all body compartments, tissues and cell types. D_2O is now used as a tracer in studies involving infants, pregnant and lactating mothers, various patient sub-groups as well healthy adults because of its excellent safety profile.

D_2O has been used to determine the kinetics of many synthetic processes by harnessing the deuterium isotope effect. It is usually administered orally and is rapidly and uniformly distributed within the body water pool, becoming incorporated into metabolic precursors producing deuterium-labelled products, including proteins (Busch, Kim et al. 2006), deoxyribonucleic acid (Yang, Matthews et al. 1984), and lipids (Lee, Bassilian et al. 1994). By analysing the

amount of these deuterium-labelled products produced over a specific period of time accurate product synthesis/breakdown rates can be determined. Advances in nuclear magnetic resonance and mass spectrometry have dramatically increased the sensitivity of detection allowing this methodology to be used for many more applications.

Early use of D_2O was in the study of skeletal muscle protein and its turnover. As technology has improved, methods have been adjusted to study specific proteins within muscle (e.g. cytosolic membrane, mitochondria and myofibrillar) and other tissues. The applications of D_2O have been extended further to investigate lipid (Bederman, Foy et al. 2009) and glucose kinetics (Katanik, McCabe et al. 2003), cell proliferation (Voogt, Awada et al. 2007), and total energy expenditure (when combined with ^{18}oxygen) (Dufner and Previs 2003).

1.2.2.2 Muscle protein breakdown

Products of skeletal muscle metabolism may be used as an index of muscle breakdown. Two metabolites in particular (creatinine and 3-methylhistidine (3-MH)), have been used to assess changes (Lukaski 1997). Creatinine is a breakdown product of protein creatine phosphate, which is principally found in muscle. Creatinine is usually produced at a fairly constant rate by the body, with increase in its urinary excretion corresponding to increases in muscle breakdown.

Endogenous excretion of 3-MH in the urine has also been suggested as an indirect measure of muscle protein breakdown (Young and Munro 1978). This amino acid is also located primarily in skeletal muscle. During muscle catabolism, released 3-MH is neither re-utilized for protein synthesis nor oxidized but, instead, is excreted in urine (Young and Munro 1978). This pathway suggests that 3-MH may also be a useful indicator of skeletal muscle mass changes. However, excretion of endogenous muscle metabolites (creatinine and 3-MH) is influenced by physical factors (physical activity, sex, age) and use of these metabolites as a measure of muscle breakdown is further complicated by the need for controlled diets (free from creatinine and 3-MH) and timed urine collections (Lukaski 1997).

1.2.2.3 Skeletal muscle mass

A variety of methods and approaches are available for estimating skeletal muscle mass. These techniques range from simple anthropometric measurements requiring inexpensive equipment to the use of sophisticated and costly instrumentation.

Anthropometry

Measurements of skin-fold thicknesses and body circumferences have been successfully used to estimate muscle mass. These approaches require measurement of a muscle group with the assumption that these measurements and subsequent changes are proportional to changes within the whole-body skeletal muscle mass. Anthropometric methods offer a low cost approach but are limited by the fact that the measurements are inaccurate estimations of regional

mass changes which are then subsequently extrapolated to estimate whole-body muscle mass (Lukaski, Mendez et al. 1981).

Measuring excreted electrolytes and muscle metabolites

The electrolyte potassium is found almost entirely within the intracellular compartment, with a large percentage (60%) distributed within skeletal muscle. Whole-body potassium may be used to estimate skeletal muscle mass using a whole-body counter (Wang, Zhu et al. 2003).

The total body potassium method has been expanded to include total body nitrogen. Prompt gamma-neutron activation analysis is used to measure total body nitrogen (Lukaski, Mendez et al. 1981). This approach assumes the potassium to nitrogen ratio of skeletal muscle and non-skeletal muscle lean mass are known and constant, thereby nitrogen levels can be used to estimate potassium levels and thus muscle mass. This method requires expensive specialist equipment and is impractical within the framework on most clinical studies.

Bioelectrical impedance

Since the advent of the first commercially available devices in the mid-1980s the bioelectrical impedance method has become popular owing to its ease of use. It works by determining the electrical impedance through various body tissues to calculate an estimate of total body water which may be used to estimate lean muscle mass. Although regional bioelectrical impedance is a simple, inexpensive technique it is unreliable in the immediate postoperative period due to constant fluctuations in individuals hydration status associated with surgery (Hanaki, Ishikawa et al. 2006).

Imaging

In contrast to other methods that indirectly assess muscle mass, imaging techniques offer unique opportunities for direct visualization and measurement of muscle. Imaging techniques provide the most accurate measurements of regional muscle mass but are hampered by unwanted exposure to radiation, expense or availability of instrumentation depending on the imaging modality used.

a. Computed tomography

Computed tomography (CT) exposes each subject to a beam of X-rays which attenuate as they pass through the body. These attenuations relate to differences in the physical density of various tissues. The differing attenuation of adipose tissue and skeletal muscle permits visual and mathematical separation of image components allowing researchers to quantify the amount of each. The main disadvantage of using CT is the radiation exposure associated with performing the scan. A CT is associated with a radiation exposure of 20 millisieverts, which is seven times the radiation exposure most people will receive within a year. Each scan is associated with a 'moderate' risk of developing cancer in the future and thus multiple scans required to quantify muscle mass changes are associated with significant risk (Wang, Zhu et al. 2003).

b. Magnetic resonance imaging

Nuclear magnetic resonance is a powerful technique providing both images and information regarding the chemical composition of tissues. As with CT, magnetic resonance imaging (MRI) can be used to assess regional and whole-body

composition. MRI uses the interaction between hydrogen atoms and the magnetic fields generated by the MRI instrument to create an image. This image details differences between the composition of various tissues within the body allowing distinction between fat and muscle. MRI is not associated with radiation exposure and is able to give detailed information for muscle mass calculations. The problems associated with use of MRI are equipment cost (between 1-2 million pounds) and its impracticality in a clinical setting. An MRI machine is claustrophobic, large and noisy. Each participant must be screened for metal either on or within their body as the machine produces a strong magnetic field.

c. Dual X-ray absorptiometry

Dual X-ray absorptiometry (DXA) exposes the patient to a beam of X-rays in order to determine bone mineral and soft tissue (fat and lean) composition (Lukaski 1993). The X-rays are attenuated in proportion to the composition and thickness of the region of the body through which the beam is passed. The attenuation is influenced by the energy of the X-rays. Specifically, soft tissues that contain water and organic compounds restrict the flux of X-rays to a lesser extent than bone. The DXA method permits assessment of whole-body and regional body composition assessment, with a particular emphasis on skeletal muscle mass. The advantage of DXA over CT and MRI is the relatively low radiation exposure (0.001millisieverts) and cost (£15,000). Again in a clinical setting it is somewhat impractical as patients would have to be moved to the machine which is housed within a specially modified room.

d. Ultrasound

B-mode ultrasound has successfully highlighted changes in body composition, and provided estimates for regional and individual skeletal muscle masses. The technique has been used in multiple studies, particularly of skeletal muscles within the upper and lower limbs. There is increasing interest in the use of ultrasound as a potential tool for exploring the changes within individual skeletal muscles by characterising the changes in muscle architecture (figure 1.10) and composition.

Successful characterisation of the changes in skeletal muscle architecture, specifically measuring variations in muscle thickness, fascicle length (fibre length) and pennation angle (angle of insertion between muscle and bone) has been achieved in numerous studies involving both healthy volunteers and patients of all ages (Narici, Binzoni et al. 1996; Maganaris, Baltzopoulos et al. 1998; Blazevich, Cannavan et al. 2007; Legerlotz, Smith et al. 2010).

The major drawback of this method is the image size available for measurements which is limited by the size of the ultrasound probe. This prevents the direct measurement of the cross-sectional area of large muscles. Researchers have overcome this obstacle by measuring changes in muscle architecture. Indeed muscle thickness and pennation angle have both been shown to significantly correlate with changes in *vastus lateralis* cross-sectional area (Narici, Maganaris et al. 2003; Morse, Thom et al. 2007). Characterisation of muscle architecture provides information regarding the number of functional units present within a muscle. Pennation angle (PA) relates to the number of sacromeres arranged in parallel whilst fascicle length (FL) relates to the number

of sarcomeres arranges in series and muscle thickness provides an estimate of the cross-sectional area at the point of measurement (POM).

Distinct advantages of B-mode ultrasound over other techniques are that it exposes the patient to no radiation at all (allowing repetition without consequences), is inexpensive (£100) and completely portable, allowing use at the bedside in postoperative patients.

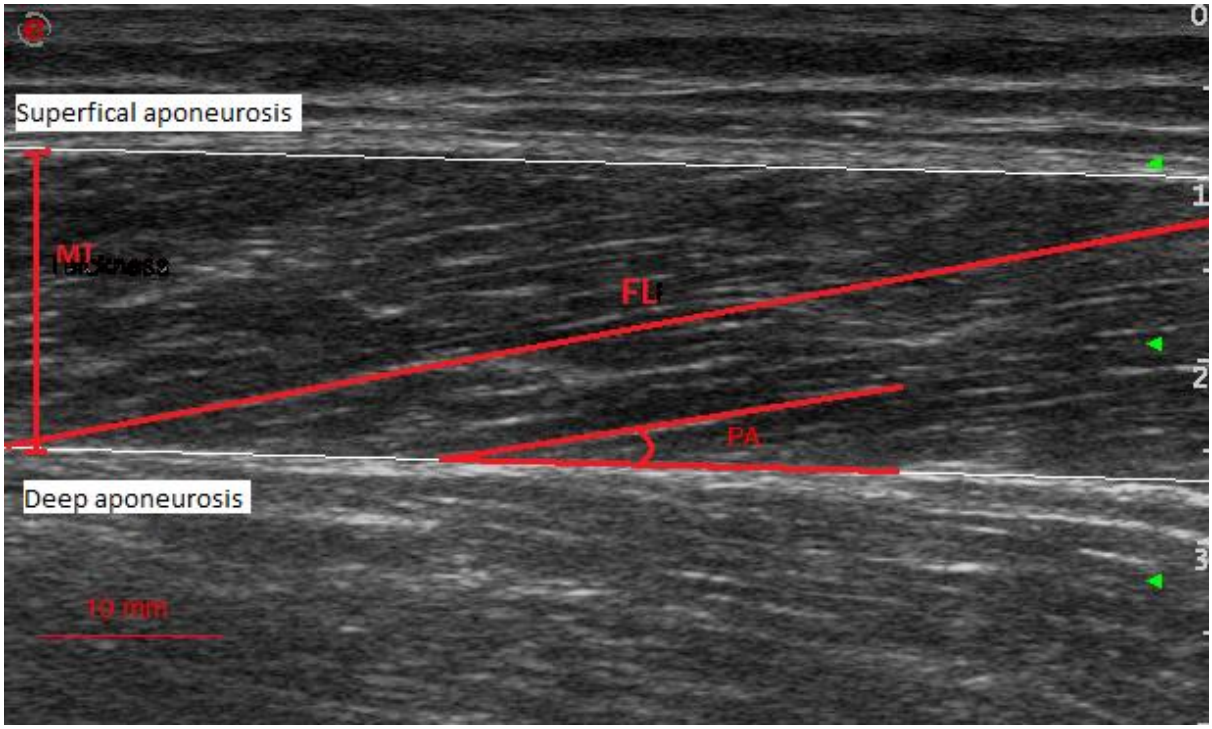


Figure 1.10 *Vastus lateralis* muscle architecture

Image captured with B-mode ultrasound of right vastus lateralis (sagittal section) to assess muscle architecture. MT- Muscle thickness; FL- Fascicle length; PA- pennation angle

1.2.3 Skeletal muscle homeostasis

The ability to maintain muscle mass in response to environmental change is governed by a fine balance between MPS and MPB. In essence, a stable muscle mass never truly exists. During the day there is constant flux between MPS and MPB. At rest, muscle protein is held in net negative balance as MPB exceeds MPS. Positive balance is achieved after feeding, when MPS is stimulated, replacing the muscle protein lost between meals (Kumar, Atherton et al. 2009). Skeletal muscle protein turns over at a rate of approximately 1–2% per day. In a healthy, weight-stable young male, this equates to 300–600 g of muscle protein broken down and resynthesized over a 24 hour period, with renewal of the body's entire skeletal muscle protein pool occurring every 3–4 months (Wall and van Loon 2013).

MPS is stimulated by three factors; feeding, via the dietary intake of both essential and non-essential amino-acids (ie. leucine and creatine); any form of exercise, and by increases in the presence of circulating anabolic hormones (ie. testosterone and insulin) (Carli and Schricker 2000; Volpi, Kobayashi et al. 2003). MPB is stimulated when the body requires utilisation of amino-acids stored within muscle protein. These amino-acids are used to produce other complex proteins or converted into glucose in response to increased energy requirements. In these circumstances muscle protein is broken down into simple amino-acids, particularly alanine, and transported to the liver via the blood stream. Here they may be used to produce new proteins or act as substrates for gluconeogenesis. These products are transported to areas of increased need (figure 1.11). This catabolic response is often seen in patients during sepsis, after trauma, or those suffering from cancer or prolonged periods of

malnourishment. In these situations protein and energy stores are often depleted whilst demands are raised.

1.2.4 Cancer cachexia

In patients suffering from colorectal cancer an overall loss of muscle mass is commonplace. The exact cause of this cachexia is multi-factorial. Studies of patients with cancer have noted MPS levels to be lower than levels in control subjects despite the presence of anabolic stimuli (either feeding or exercise) (Coss, Bohl et al. 2011). Added to the inability to stimulate MPS, inflammatory mediators released by cancer cells and damaged tissues stimulate MPB (Williams, Phillips et al. 2012). The stimulation of MPB releases substrates locked within muscle proteins required to promote continued tumour growth and tissue repair respectively.

These changes in MPS and MPB are controlled cytokines released by the tumour and surrounding cells, producing a cancer-associated systemic inflammatory response (Richards, Roxburgh et al. 2012). Finally, the exact anatomical site of the tumour may also affect total muscle mass. For example upper gastrointestinal tumours may alter a patient's ability to feed or absorb nutrients which suppresses anabolic stimulation of MPS.

Evidence suggests severe cachexia is associated with treatment toxicity, poor functional status and decreased overall survival in patients suffering from gastrointestinal cancers (Prado, Lieffers et al. 2008; Peng, van Vledder et al. 2011; van Vledder, Levolger et al. 2012). In patients undergoing elective

surgery for colorectal cancer, preoperative loss of muscle mass is also associated with increased incidence of postoperative infection and delayed recovery (Lieffers, Bathe et al. 2012).

1.2.5 Sarcopenia

As we age our ability to maintain a constant muscle mass diminishes. This phenomenon has been labelled sarcopenia. In essence, sarcopenia is the result of a steady mismatch between MPS and MPB over time. Interestingly, at rest neither MPB nor MPS have been found to differ between young and older individuals (Narici and Maffulli 2010).

However, differences do exist in response to feeding and exercise. Regarding MPS, studies have shown a blunted response to anabolic stimuli in older individuals. Compared to younger controls, older people show a lower increase in MPS in response to amino acid feeding (~−40%) and also in response to exercise (~−30%) (Cuthbertson, Babraj et al. 2006).

Similarly, despite the absence of differences in MPB at rest, important differences have been discovered in response to feeding. The inhibition of MPB by insulin in response to feeding is also blunted in older individuals when compared to younger adults (Narici and Maffulli 2010). Unsurprisingly, colorectal cancer patients are exposed to several external and internal influences which may alter the balance between MPS and MPB particularly, cancer cachexia, sarcopenia, variations in nutritional status, inability to exercise and the surgical stress response (SSR) (Carli and Schricker 2000).

1.3 THE SURGICAL STRESS RESPONSE

1.3.1 Overview

The stress response is a basic survival mechanism to overcome injury. The aim is to increase the amount of energy generating substances at our disposal in order to overcome the physical and metabolic insults caused by injury.

As early as in 1932, Cuthbertson described in detail the metabolic responses of four patients with lower limb injuries (Cuthberson 1932). Through this work, he introduced the terms 'ebb' and 'flow' to describe the initial decrease in metabolic activity due to injury and subsequent delayed increases which are associated with repair.

After early work on the stress response to accidental injury, attention turned towards surgical trauma and the term 'surgical stress response' (SSR) became popular. Originally, the SSR was ascribed to increases in the secretion of catabolic hormones (catecholamines, cortisol, glucagon) and concomitant decreases in the secretion of, or increased resistance towards, the anabolic hormones (insulin) (Carli and Schricker 2000).

Over time, research has shown the SSR to be rather more complex (figure 1.11). The SSR is an interconnected mixture of metabolic, hormonal, autonomic, haematological and immunological responses which activate the sympathetic nervous system (Desborough 2000).

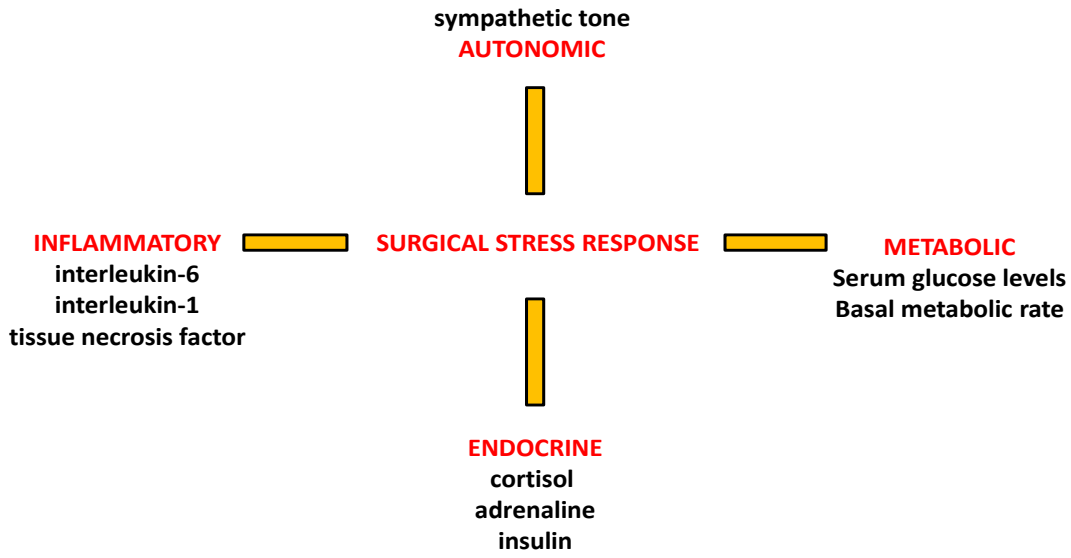


Figure 1.11 The surgical stress response

Major effects of activating a surgical stress response.

The SSR is activated by tissue damage (and pain) via afferent neuronal impulses emanating from the site of injury. These travel along sensory nerve roots through the dorsal root of the spinal cord, and up to the brain (medulla) to activate the hypothalamus (Desborough 2000). This stimulates the release of several hormones via the hypothalamic-pituitary axis and also activates the sympathetic nervous system directly (Table 1.8 & 1.9). This initial stimulus is modulated by cytokines, especially interleukin-6 (IL-6) and tissue necrosis factor, which are released by leucocytes and endothelial cells present at the site of surgical injury (Sheeran and Hall 1997; Helmy, Wahby et al. 1999).

Organ	Effect
Heart	Increased heart rate Increased force of contraction
Blood vessels	Constriction
Digestive tract	Decreased motility Inhibition of exocrine secretions
Liver	Glycogenolysis Lipolysis
Lungs	Dilation of bronchioles
Adrenal gland	Stimulates secretion of adrenaline
Pancreas	Inhibition of insulin secretion Stimulates glucagon secretion

Table 1.8 Effects of sympathetic nervous system stimulation upon major organs involved in the surgical stress response.

Endocrine gland	Hormone	Action	Secretion
Anterior pituitary	ACTH	Increased production and release of corticosteroids	Increased
	GH	Increases muscle mass Promotes lipolysis Increases protein synthesis Reduces liver uptake of glucose Promotes gluconeogenesis in the liver Stimulates the immune system	Increased
Posterior pituitary	Vasopressin	Increases peripheral vascular resistance Stimulates water retention	Increases
Adrenal	Cortisol	Promotes gluconeogenesis in the liver Suppress the immune system Stimulates fat and carbohydrate metabolism Inhibits collagen formation Decreases amino acid uptake by muscle Inhibits protein synthesis	Increases
	Aldosterone	Increases sodium and water retention	Increases
Pancreas	Insulin	Reduces blood glucose levels Transports glucose into tissues	Decreases
	Glucagon	Increases blood glucose levels Releases glucose from tissues	Increases
Thyroid	Thyroxine	Regulates of rates of metabolism	Decreases

Table 1.9 Principle endocrine surgical stress responses.

ACTH- corticotrophin; GH- growth hormone.

1.3.2 Cytokines

Cytokines are low-molecular-weight proteins which include the interleukins and interferons. They are produced by activated leucocytes, fibroblasts and endothelial cells in response to tissue injury and have a major role in mediating immunity and inflammation (Sheeran and Hall 1997). Cytokines activate surface receptors of target cells and influence protein synthesis within the cell. After major surgery, the main cytokines released are interleukin-1 (IL-1), tumour

necrosis factor-α (TNF-α) and IL-6 (Desborough 2000). Initially IL-1 and TNF-α are released from activated macrophages and monocytes within damaged tissues. This stimulates the production and release of more cytokines, in particular, IL-6, the main cytokine responsible for inducing the systemic changes known as either the acute phase or surgical stress response (Sheeran and Hall 1997).

Within 30–60 min after the start of surgery, IL-6 concentration increases to a maximum at 24 hours and remains elevated for 48-72 hours postoperatively (Desborough 2000). Cytokine production directly reflects the degree of tissue trauma with the largest increases in IL-6 occurring after open orthopaedic, vascular and colorectal surgery (Desborough 2000).

1.3.3 The acute phase response

The systemic changes occurring after tissue injury and IL-6 release are known as the 'acute phase response'. The principle features of this response are pyrexia and the production of acute phase proteins within the liver. These acute phase proteins act as inflammatory mediators, anti-proteinases and scavengers and include C-reactive protein (CRP). The increase in serum concentrations of CRP follows changes in IL-6 concentrations. Production of other proteins in the liver, for example, albumin and transferrin, decreases during the acute phase response (Desborough 2000). Concentrations of circulating cations such as zinc and iron also decrease, partly as a consequence of the changes in the production of their transport proteins (Sheeran and Hall 1997).

1.3.4 Negative feedback systems & control

Postoperatively, cytokine release may augment pituitary ACTH secretion and subsequently increase the release of cortisol. A negative feedback system exists, with glucocorticoid release inhibiting further localised cytokine production. Studies have shown the cortisol responses to surgery are sufficient to reduce IL-6 concentrations in the first few postoperative days (Jameson, Desborough et al. 1997). In this way the body can augment its response to surgery using this negative feedback model. Although it seems the stress response developed to allow injured animals to survive by catabolizing their own stored body fuels, it has long been argued that this response is unnecessary in modern surgical practice (Desborough 2000).

1.3.5. Surgical stress response & skeletal muscle modulation

The SSR has a major catabolic effect upon skeletal muscle after abdominal surgery (figure 1.12). Protein catabolism is stimulated by increased levels of cortisol. Predominantly skeletal muscle is broken down, but some visceral muscle protein is also catabolized to release the constituent amino acids (Desborough 2000). As above, the amino acids may be further catabolized for energy or used in the liver to produce new protein, particularly acute-phase proteins. The liver also converts amino acids into other substrates, glucose, fatty acids or ketone bodies. Protein catabolism results in marked postoperative weight loss and muscle wasting. The extent of muscle protein loss following colorectal surgery depends upon a variety of factors including: the patient's nutritional status, presence of underlying disease processes (cancer cachexia and sarcopenia), type of anaesthesia administered, and type of surgery performed (open or laparoscopic).

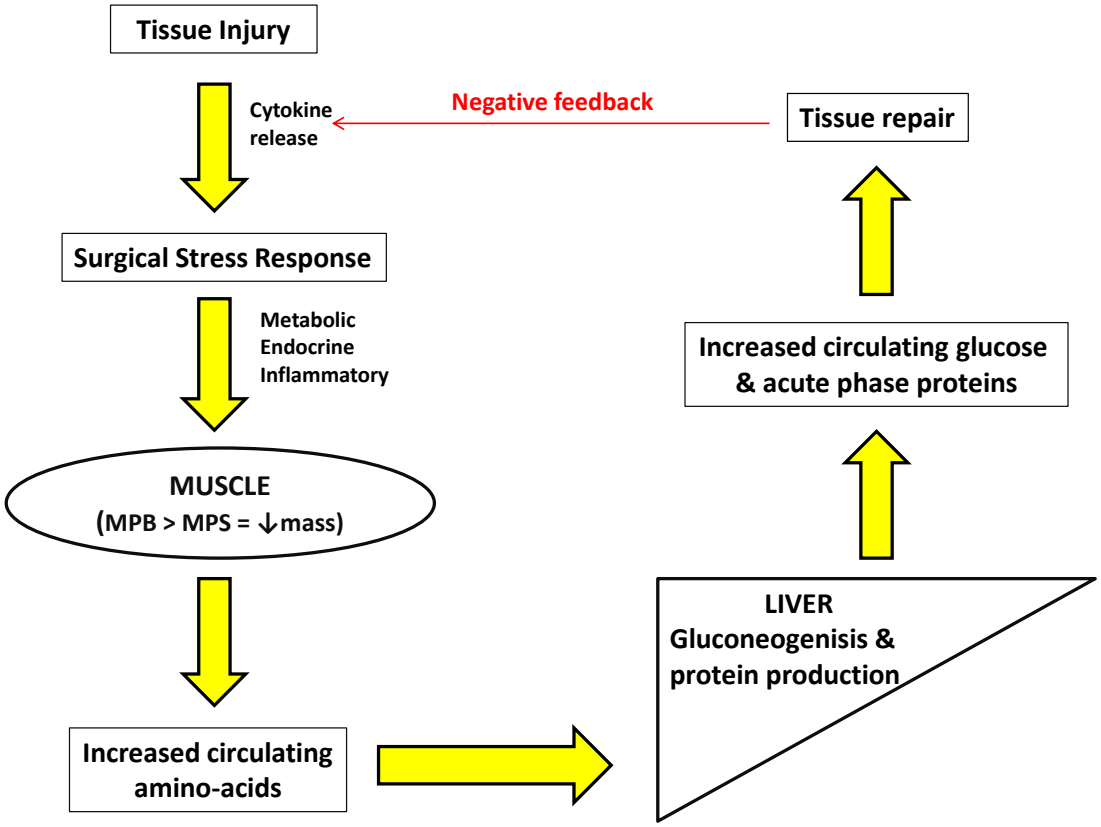


Figure 1.12 Mechanisms contributing to skeletal muscle modulation

Muscle protein breakdown (MPB); Muscle protein synthesis (MPS)

1.3.6 Evidence for postoperative muscle modulation

Numerous studies have proven the association between colorectal surgery and a degree of muscle modulation causing an overall loss of skeletal muscle mass (Jensen and Hessov 1997; Kissmeyer-Nielsen, Jensen et al. 1999; Henriksen, Jensen et al. 2002; Houborg, Jensen et al. 2006; Norager, Jensen et al. 2006). These studies have used a variety of different techniques to measure the changes occurring over the first few months after surgery. Studies employing isotope-labelled, non-radioactive amino acids have allowed us to quantify these changes in whole-body protein synthesis and breakdown after surgery.

Several forms of major abdominal surgery have been associated with losses of between 0.5-1.5 kg of lean tissue mass postoperatively (Williams, Phillips et al. 2012). In addition to the catabolic effects of the SSR, driven by increases in MPB in order to replenish spent energy stores, studies have also shown MPS to be unchanged at the end of a major surgery and significantly depressed in the postoperative recovery phase (Tjader, Essen et al. 2004). Indeed postoperative measurements of MPS noted that at 4 hours it is depressed and continues to decrease for between 1and 3 days after surgery depending upon the operation performed (Essen, McNurlan et al. 1993; Petersson, Hultman et al. 1995; Carli and Halliday 1997; Caso, Vosswinkel et al. 2008). This stimulation of MPB and depression of MPS within the postoperative period produces a surgery-induced muscle atrophy which is most pronounced between 2 and 4 weeks after surgery (Abdiev, Kodera et al. 2011). Thereafter, depending upon the surgery performed, muscle mass begins to stabilise and may recover slowly as MPS returns to normal levels (Streat and Hill 1987; Christie and Hill 1990; Hill, Douglas et al. 1993).

1.3.7 Postoperative muscle modulation & surgical outcomes

There is strong evidence supporting a direct relationship between poor body composition and increased morbidity and mortality. Preoperative obesity and cachexia (sarcopenic and cancer-related) are associated with poor general health and increased surgical risk.

Postoperative changes in body composition, in particular a reduction in skeletal muscle mass, are also associated with increased morbidity and mortality. Although, the exact weight loss needed to lead to deterioration in physical function is not known, it has been speculated that in elective surgical patients weight loss has to exceed at least 4 kg before a reduction in physical performance can be anticipated in an average patient (Bach Jensen and Hessov 2000).

To note the effects of surgery alone on muscle modulation and outcomes, we need to review studies where patients did not suffer from any weight loss or change in body composition preoperatively. Over a ten year period a Danish group conducted 5 randomised controlled trials assessing postoperative changes in physical performance after open colorectal surgery (both benign and malignant). The amalgamated data from these studies concluded that open colorectal surgery was associated with loss of lean muscle mass which in-turn was associated with significant increases in length of stay, long term fatigue and declined physical performance (Jensen, Houborg et al. 2011). Recently in 2012, van Venrooij et al. studied 29 patients undergoing cardiac surgery and again noted postoperative loss of muscle mass was again associated with decreased vitality and increased length of stay (van Venrooij, Verberne et al. 2012).

In the short term, excessive muscle modulation (combining pre and postoperative loss of muscle mass) may lead to complications including delayed wound healing, compromised immune function, pneumonia, fatigue and diminished strength producing prolonged convalescence and increased morbidity (Windsor and Hill 1988).

1.4 PRESERVATION OF MUSCLE MASS AFTER COLORECTAL SURGERY

Losses of skeletal muscle mass must be minimised when embarking on major surgery to contribute to better outcomes. Modifiable risk factors are: nutrition, exercise, and the SSR. By influencing these three areas clinicians may be able to restore the balance between MPB and MPS.

1.4.1 Preoperative interventions

The period of time between diagnosis and undergoing surgery for colorectal cancer is widely variable. Recently a Canadian research group has attempted to improve surgical outcomes through use of preoperative programs aimed at improving functional capacity and coined the term 'prehabilitation'. To date there have been three randomised controlled trials using colorectal cancer patients. The first, by Kin et al., noted exercise improved pre-operative exercise tolerance only (Kim do, Mayo et al. 2009). The second, by Carli et al., introduced a four week exercise regime of either walking or riding a bicycle. It noted improved physical function both pre- and postoperatively, however the intervention did not affect outcomes or length of stay (Carli, Charlebois et al. 2010).

A third trial, by Li et al., implemented a tri-modal prehabilitation program including regular exercise, nutrition supplementation (whey protein) and the incorporation of techniques to reduce anxiety (Li, Carli et al. 2013). They found the 1 month program improved functional walking capacity and was associated with better postoperative recovery. None of these studies investigated improvements in muscle mass or modulation, however, theoretically exercise regimes and nutritional supplementation may improve recovery by redressing the preoperative imbalances between MPB and MPS responsible for cancer cachexia.

1.4.2 Perioperative interventions (Enhanced recovery programs)

Interventions used to improve nutrition and mobility whilst dampening the SSR are interlinked in the perioperative period (figure 1.13). Several strategies have been designed to improve postoperative recovery by minimizing muscle mass losses and all are incorporated into modern enhanced recovery programs (ERP).

ERPs first appeared in the 1990's and quickly become an important component in the perioperative management for colorectal, vascular, thoracic, gynaecological, breast and urological surgery (Kehlet 1997; Tovar, Roethe et al. 1998; Podore and Throop 1999; Wind, Polle et al. 2006; Lv, Wang et al. 2010; Arsalani-Zadeh, ElFadl et al. 2011). The main focus of these programs is to blunt the psychological and physiological responses to major surgery (Fearon, Ljungqvist et al. 2005). Implementation of such a program in surgery for colorectal cancer is known to reduce complications and length of hospital stay, improve cardiopulmonary function, reduce muscle mass loss, reduce postoperative ileus and hasten resumption of normal activities (Kehlet 2008; Eskicioglu, Forbes et al. 2009; Lassen, Soop et al. 2009).

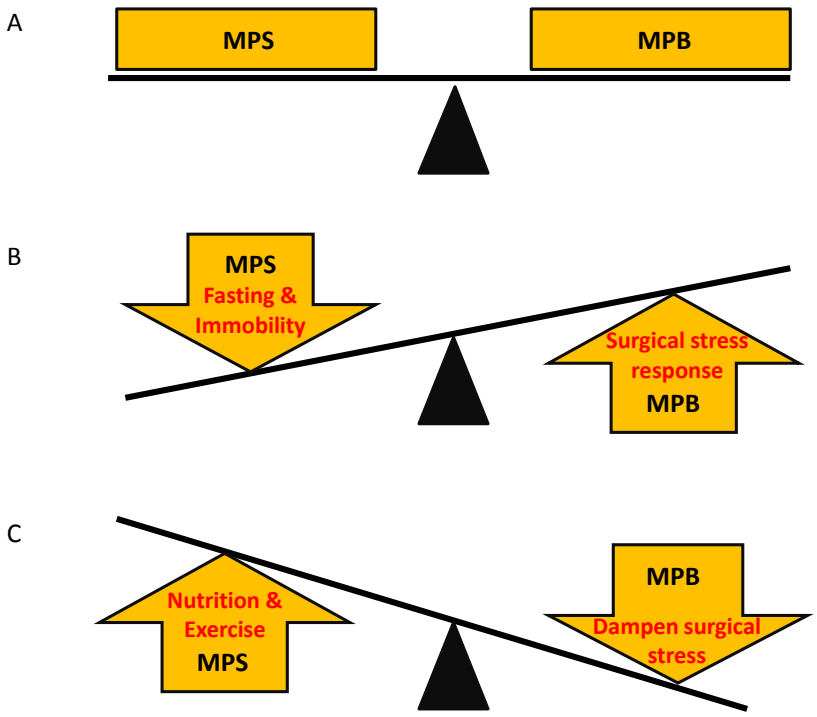


Figure 1.13 Principles of an enhanced recovery program on skeletal muscle modulation

A- Normal muscle homeostasis (MPS = MPB); B- Effects of surgery upon MPS and MPB (MPS<MPB); C- Effects of enhanced recovery program upon MPS and MPB (MPS>MPB). Muscle protein synthesis (MPS); Muscle protein breakdown (MPB).

1.4.2.1 Fasting

Consumption of protein is key to stimulating MPS. In addition, there is little evidence to support the practice of fasting patients from midnight before surgery in order to avoid pulmonary aspiration (Crowe, Dennison et al. 1984). Preoperative fasting increases metabolic stress, causing hyperglycaemia and increased insulin resistance (Thorell, Nygren et al. 1999). Modern enhanced recovery programmes utilise shortened fluid fasting regimens. There is no

evidence to suggest that these shortened fasting regimes increase the risk of aspiration, regurgitation or morbidity compared to standard fasting from midnight (Brady, Kinn et al. 2003). A shortened preoperative fasting time has been shown to decrease insulin resistance and subsequently reduce muscle mass losses (Ljungqvist and Soreide 2003). In line with the current evidence, guidelines introduced by the Royal College of Anaesthetists therefore allow patients' to take solids up to 6 hours preoperatively and clear fluids for up to 2 hours.

1.4.2.2 Carbohydrate loading

The relationship between SSR and insulin is central to responses in the muscle. The SSR reduces insulin secretion and increases peripheral resistance towards it (Thorell, Nygren et al. 1999). The net effect is to increase circulating levels of glucose and so that it is available for use in vital organs or tissues (Soop, Carlson et al. 2004; Mathur, Plank et al. 2008). Hyperglycaemia and insulin resistance contributes to increased length of stay, poor wound healing and increased risk of infective complications (Thorell, Nygren et al. 1999). Insulin resistance is positively related with more major surgery. Postoperative hyperglycaemia which is tightly controlled with intensive insulin therapy has been shown to reduce mortality and morbidity by a third in the intensive care setting (van den Berghe, Wouters et al. 2001). The degree of postoperative insulin resistance may be reduced by ensuring adequate analgesia, minimising time when patients are not feeding orally and with the use of preoperative carbohydrates (Crowe, Dennison et al. 1984; Greisen, Juhl et al. 2001).

Preoperative loading with oral carbohydrate is known to alter postoperative insulin resistance, reduce protein losses, and preserve skeletal muscle mass

(Nygren, Soop et al. 1998; Svanfeldt, Thorell et al. 2007). It may also improve overall nutrition by reducing thirst, hunger and promotes gastrointestinal function (Noblett, Watson et al. 2006).

1.4.2.3 Anaesthesia and analgesia

Pain is regarded as a key trigger for the SSR and its reduction depends upon close involvement with the anaesthetist in formalising a postoperative pain management regime (White, Kehlet et al. 2007). Historically, high dose opiates were used as postoperative analgesia, with both morphine and fentanyl shown to suppress the endocrine and metabolic responses to surgery that stimulate muscle mass losses (Giesecke, Klingstedt et al. 1994). Despite the excellent analgesic properties of opiates, unwanted side effects including, excessive sedation, respiratory depression, constipation and prolonged ileus have limited their effectiveness in the postoperative phase in contribution to areas other than analgesia per se. A non-functioning gastrointestinal tract may exacerbate muscle mass losses as the body is unable to absorb much of the protein required to initiate and facilitate MPS. Excessive sedation also has a negative impact on muscle mass as subsequent delays in postoperative mobilisation exacerbated losses through non-use (Drummond, Dickinson et al. 2012). Recently newer shorter-acting opiates (alfentanil and sufentanil) dampen stress responses when administered in small doses and are associated with fewer unwanted effects. The use of COX-II inhibitors is well established with usage shown to reduce opiate consumption and therefore should form part of multimodal pain management within enhanced recovery (Sim, Cheong et al. 2007).

Epidural analgesia is commonly used to reduce total doses of general analgesia required during surgery and to decrease the amount of afferent pain fibre

stimulation during and after colorectal surgery, therefore dampening the SSR with fewer sedating side effects (Jorgensen, Wetterslev et al. 2000; Hong, Yang et al. 2011). If sited before the start of surgery release of stress hormones is reduced (Marret, Remy et al. 2007). Epidural administration of opiates alone does not reduce the SSR, whilst blockade of both nociceptive and non-nociceptive pathways has been found to decrease MPB and prevent reductions in MPS after surgery as long as the epidural remains effective in the postoperative period (Carli, Webster et al. 1991; Carli and Halliday 1997). Epidural blockade does not decrease muscle mass losses when perioperative calorie intake is low, supporting the notion that the protein-sparing influence of epidural blockade requires the patient to receive adequate nutrition (Schricker, Wykes et al. 2000). The major problem with epidural analgesia is immobility in the immediate postoperative period due to the presence of the epidural catheter within the patient and the infusion pump at the bedside and inadvertent motor blockade. Prolonged bed rest (> 2 days) is associated with significant muscle atrophy (due to decreases in MPS), therefore early postoperative ambulation and mobilization is essential in order to contribute to the anti-catabolic effect of epidural analgesia (as long as it is removed once the patient is able to tolerate oral analgesia) (Ferrando, Lane et al. 1996).

To address immobility associated with epidural use, whilst maintaining the positive effects of adequate perioperative analgesia, many anaesthetists now administer a '*transversus abdominis* plane' (TAP) blockade. Indeed for abdominal surgery below the level of the umbilicus, symmetrical sensory blockade with local anaesthetic alone (extending from T4 to S5 dermatomes) also attenuates the SSR and has been shown to facilitate a rapid return in the patient's mobility if complemented with patient controlled analgesia (Johns, O'Neill et al. 2012). More recently anaesthetists have introduced a single bolus

of spinal analgesia before induction of general anaesthesia. Here a mixture of opiate and local anaesthetic is infiltrated around the spinal cord to temporally block all sensation below the injection site. This method does not require bed rest and may provide postoperative analgesia for up to 48 hours prior to use of oral agents thereby improving recovery times (Levy, Scott et al. 2011).

1.4.2.4 Anti-emetics

Avoidance of postoperative nausea and vomiting (PONV) is extremely important. Inability to maintain an oral intake can cause major problems with analgesia and nutrition. The ERAS (enhanced recovery after surgery) Group recommends risk stratification for PONV using the *Apfel* scoring system, with prophylaxis given for patients at moderate or high risk. It recommends the use of dexamethasone at induction or a 5HT3 receptor antagonist at the end of surgery in moderate risk patients, and total intravenous anaesthesia with dexamethasone at induction and either a 5HT3 receptor antagonist, or metoclopramide near the end of surgery in high risk patients.

A subsequent Cochrane review of antiemetic prophylaxis has not shown a beneficial effect of one agent over another (Carlisle and Stevenson 2006). An on-going multicentre randomised control trial (Acronym: DREAMS) is currently attempting to provide information regarding best antiemetic practice in colorectal surgery.

1.4.2.5 Nutrition

As above, PONV and/or a non-functioning gastrointestinal tract can significantly decrease a patient's ability to absorb protein and stimulate MPS. Assessment and treatment of poor postoperative nutrition is an essential component of

enhanced recovery. Correction of either pre or postoperative deficiencies may require additional enteral and/or parenteral supplements.

1.4.2.6 Minimally invasive surgery

Minimally invasive techniques have resulted in smaller incisions and reduced tissue damage during surgery. This has a potential effect upon the catabolic responses to surgery and ultimately recovery.

Patients undergoing laparoscopic abdominal surgery (cholecystectomy, colonic resection, appendectomy) have reduced levels circulating of inflammatory mediators (interleukin-6, C-reactive protein and tissue necrosis factor) compared with corresponding open procedures (Kehlet and Nielsen 1998). A recent study by *Veenhof et al*. (Veenhof, Sietses et al. 2011) reported reduced levels of circulating IL-6 two hours post laparoscopic TME for rectal cancers compared with open procedures. Furthermore, reduced losses of lean muscle mass following laparoscopic upper gastrointestinal surgery have reported compared with open operations (Kiyama, Mizutani et al. 2005; Abdiev, Kodera et al. 2011). Attenuated SSR is thought to be partially responsible for the improved recovery seen in patients undergoing laparoscopic surgery. Other postoperative responses, such as impairment of muscle exercise performance, hypoxemia, and intestinal paralysis are also improved by the use of laparoscopic techniques (van Bree, Vlug et al. 2011).

1.4.3 Postoperative interventions

Rehabilitation and nutrition

Exercise regimes are potentially beneficial, as surgery and immobility are important risk factors for thromboembolic events and chest infection, both associated with high levels of mortality. Exercise also stimulates MPS and may improve postoperative outcomes (Haines, Sinnamon et al. 2010). Skeletal muscle undergoes modulation in response to exercise. Both resistance and endurance training have shown to help increase muscle mass in healthy volunteers as long as a steady supply of protein is available for MPS to occur (Tipton, Borsheim et al. 2003). During exercise there is an initial depression in MPS, however after the exercise period is completed there is a progressive elevation in MPS which ultimately plateaus, remaining elevated for a considerable length of time. The effects of exercise on MPB are still unclear (Kumar, Atherton et al. 2012).

However, there has only been one randomised control trial to date looking at the effect of offering postoperative nutrition and exercise to colorectal patients to ameliorate muscle mass losses and improve outcomes. *Houborg et al*., noted that postoperative physical training and protein supplementation had little effect on body composition or surgical outcomes for patients who had undergone colorectal cancer surgery (Houborg, Jensen et al. 2005). Although robust, this study was performed on a healthy and relatively young Danish population with no patient suffering from any form of preoperative cancer cachexia (when reviewing body mass index values). Potentially surgery may have greater detrimental effects upon elderly patients, particularly those suffering from preoperative cachexia.

1.5 Summary

At present surgery is the only curative treatment available to patients diagnosed with colorectal cancer. As our understanding of the body's response to colorectal surgery has developed so too has our appreciation of the importance of muscle modulation and the need to control mechanisms affecting MPB and MPS to improve functional outcomes. Enhanced recovery programs and laparoscopic surgery have markedly improved recovery however a significant proportion of patients still show signs of skeletal muscle mass losses and delayed recovery, or are unable to undergo minimally invasive surgery.

Chapter 2

Aims & objectives

2.1 AIMS

This thesis aims to systematically investigate skeletal muscle modulation and functional recovery after potentially curable surgery for colon cancer comparing open and laparoscopic surgical techniques.

To fulfil these aims two separate studies were conducted. The first, entitled 'skeletal muscle modulation after colorectal cancer resection' (SMMAC) is a prospective longitudinal observation study in which patients recruited preoperatively were assessed regularly for 6 months post surgery. The primary endpoint for this study is a functional outcome, hand grip strength. A previous pilot study noted a relatively larger decrease in postoperative hand grip strength in patients undergoing open compared to laparoscopic surgery, and this data was used for power calculations in order to estimate sample size. An overview of the study and demographic data is presented in chapter 3 with subsequent chapters presenting data collected for the surgical stress response, muscle modulation and functional recovery respectively.

A second study was conducted to assess long-term functionality 1-year after potentially curative colorectal cancer surgery. This is retrospective questionnaire based study focusses upon return to work as an outcome measure relating to full functional recovery and is presented in chapter 7.

Chapter 3

Skeletal muscle modulation after colorectal cancer resection: Study overview

3.1 STUDY TITLE

Skeletal muscle modulation after colon cancer resection (Acronym: SMMAC)

3.2 STUDY DESIGN

This is a prospective observational longitudinal cohort study comparing recovery after potentially curable colonic cancer resection performed by open and minimally invasive techniques.

To compare the effect of surgical technique upon skeletal muscle modulation and functional recovery every attempt must be made to eliminate bias. Several preoperative factors potentially affect recovery including tumour site, tumour stage and patient age (Bhalla, Williams et al. 2014). When considering tumour site, evidence suggests patients diagnosed with rectal cancer have a significantly longer recovery phase due to either the presence of a stoma or from the increased prevalence of abnormal bowel habit or urinary function after surgery (Rodriguez-Bigas, Chang et al. 2007). Colon cancer patients do not typically require a temporary or permanent stoma. Previous studies in our centre highlight a low rate of laparoscopic rectal resection, making an equal allocation of patients difficult (regarding tumour site and surgical technique) (Bhalla, Williams et al. 2014). Therefore this study was specifically designed to recruit patients diagnosed with colonic adenocarcinoma only.

Bowel function after colon resection generally returns to normal or near normal except in situations where extended resection (subtotal or total colectomy) is performed with the resultant potential for diarrhoea and the need for

antidiarrheal medication (Rodriguez-Bigas, Chang et al. 2007). For this reason we decided not to recruit patients having sub-total colectomy to eliminate a significantly different procedure as a potential source of bias. Our colonic surgery group does not include any patients where extended resection (subtotal or total colectomy) occurred.

Tumour stage is also an important source of bias. Advanced tumour stage requires additional preoperative or postoperative adjuvant chemotherapy both of which have been shown to delay recovery and reduce quality of life scores in colorectal cancer patients (Mols, Thong et al. 2009; Earle, Chretien et al. 2010). We retrieved information relating to tumour stage and use of post-operative chemotherapy to minimise this potential source of selection bias.

3.3 AIM

To review recruitment processes and compare demographic data, complication rate and length of stay of patients undergoing potentially curable minimally invasive surgery for colon cancer with patients undergoing open surgery.

3.4 METHODS

3.4.1 Ethics

The study was sponsored by the University of Nottingham (reference no. 12134). Ethical approval was gained from the Nottingham Research Ethics Committee I (NRES reference: 13/EM/0031) and approved by the Royal Derby Hospitals NHS Trust (see appendix I).

3.4.2 Sample size calculations

This study was powered to detect a greater reduction in handgrip strength for patients undergoing open rather than laparoscopic surgery. A pilot study performed by our research group noted hand grip strength in patients undergoing potentially curable surgery for colorectal cancer maximally diminished on the second postoperative day. This drop was 20% greater in patients undergoing open compared with minimally invasive surgery. Using this data with a 'P' value set at 0.85 and one-tailed analysis (GPower v3.1 Heinrich-Heine-Universität, Düsseldorf, Germany) our study was powered for n=24 patients. Assuming a 25% drop-out rate, ethical approval was gained for recruitment of 30 patients in each group (n=60 total).

3.4.3 Recruitment

Information for potential participant fulfilling the inclusion criteria was gathered from the Derbyshire NHS Trust's colorectal multidisciplinary team meeting (Table 3.1). These patients were approached after an outpatient meeting with their assigned surgeon. Participant information packs containing details of the study were provided to potential volunteers and any questions answered (see appendix II). Formal consent was gained during a subsequent outpatient appointment prior to surgery (pre-operative assessment clinic).

Inclusion	**Exclusion**
Colonic adenocarcinoma	Pregnancy
No metastasis	Immunomodulating medication
No contraindication for enhanced recovery	Warfarin

Table 3.1 Inclusion/exclusion criteria for SMMAC study

3.4.4 Study timeline

The timeline for this study is shown in figure 3.1. Patients underwent various assessments at each time point (Table 3.2). All patients followed Royal Derby Hospitals NHS Trust's guidelines for enhanced recovery after colorectal surgery (see appendix II). Detailed methodology for every part of the study, together with corresponding results are presented within Chapters 4, 5 and 6.

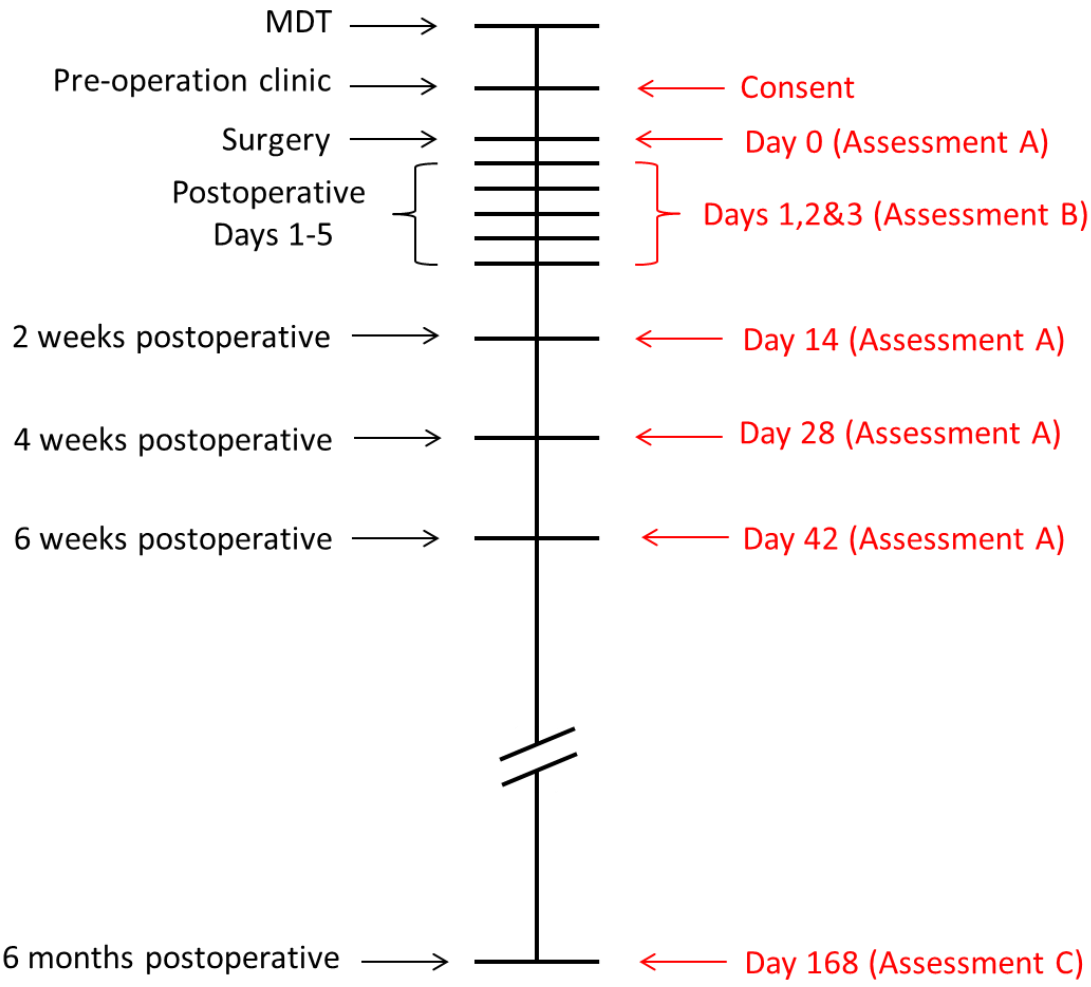


Figure 3.1 SMMAC study timeline.

The patient's clinical journey is depicted on the right side in black whilst SMMAC study days are depicted on the left side in red.

Study day assessments		
Assessment A	**Assessment B**	**Assessment C**
SSR GLR IL-6	**SSR** GLR IL-6	**SSR** GLR IL-6
Skeletal muscle modulation Muscle architecture MPS	**Skeletal muscle modulation** Muscle architecture	**Skeletal muscle modulation** Muscle architecture
Recovery Pain Hand grip EQ5d5l DASI	**Recovery** Pain Hand grip	**Recovery** Pain Hand grip EQ5d5l DASI

Table 3.1 Study day assessments for the SMMAC study.

SSR- surgical stress response; GLR- granulocyte lymphocyte ratio; Interleukin-6- IL-6; MPS- muscle protein synthesis; EQ5d5l- EuroQuol 5-dimension questionnaire (English language edition); DASI- dukes activity status index

3.4.5 Statistical analysis

Data was analysed on completion of the study using SPPS version 21 (IBM, New York, US) with significance levels set at 0.05. Data was analysed in-house with advice provided by Dr Lynda Cochrane, medical statistician, Division of Population Health Sciences, Medical Research Institute, University of Dundee. Independent t-tests were used to compare independent parametric data; paired t-tests for paired parametric data, Mann-Whitney U and Kruskal-Wallis tests for non-parametric ordinal and categorical data respectively. Chi-squared tests were used for nominal data and Pearson's product-moment coefficient was calculated

to assess correlation (as data was collected within an interval scale and a linear relationship was suspected).

3.5 RESULTS

3.5.1 Recruitment data

A flow-diagram summarising the recruitment process is shown in figure 3.2. Every participant who consented to the study proceeded to surgery. Fifty-four patients were recruited and the groups evenly split. Four patients undergoing laparoscopic surgery had their operative technique converted to open surgery due to technical difficulties associated with visualisation of the segmental colonic pedicle. These patients were analysed within the open group due to the presence of a laparotomy incision. Each group was further sub-divided by the anatomical site of the tumour to eliminate potential bias.

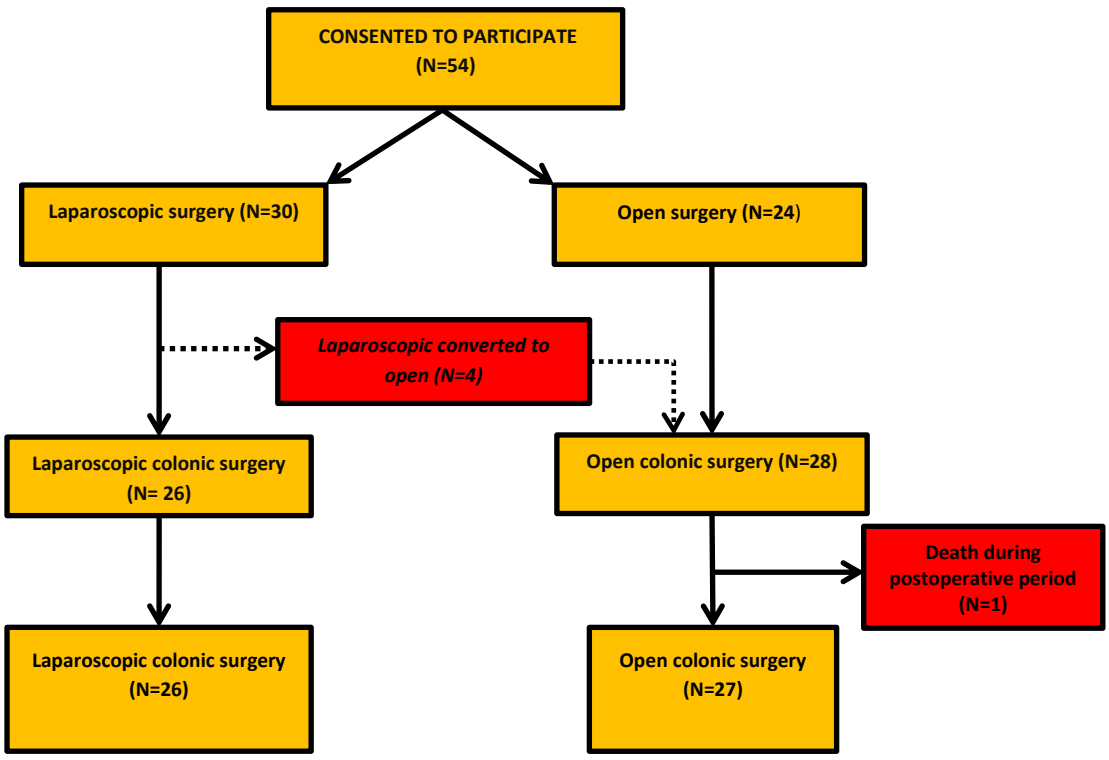


Figure 3.2 Flow-diagram for SMMAC

3.5.2 Patient co-operation

Patient compliance on each particular study day is shown in figure 3.3. Each recruited patient had data collected preoperatively and throughout their in-patient stay. Twenty-two open and 21 laparoscopic patients returned at day 14. At day 28, 23 open and 21 laparoscopic patients underwent assessments. Twenty-three open surgery and 22 laparoscopic patients returned at day 42 whilst 24 open and 23 laparoscopic patients returned at 6 months.

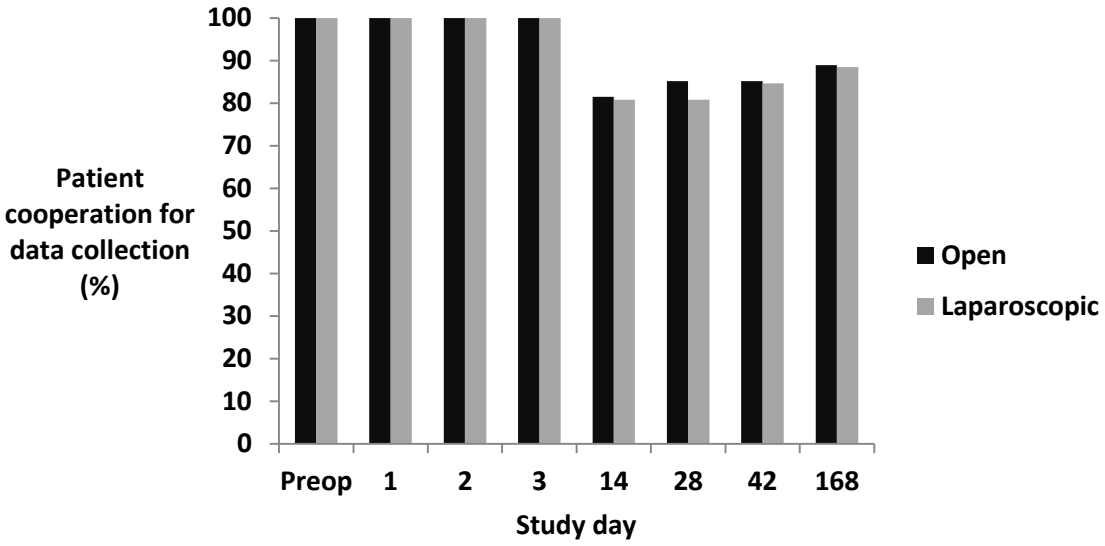


Figure 3.3 Patient co-operations for data collection during SMACC study

3.5.3 Colonic tumour demographics

Demographic data allowing comparison between open and laparoscopic colonic resection groups is presented in table 3.3. No statistical difference exists between groups when reviewing data for age, sex, BMI, tumour stage and blood loss. Significant differences are noted for operation time (open surgery 141.6±10.2 v laparoscopic surgery 182.5±10.83; $p<0.05$) and length of stay (open surgery 7 v laparoscopic surgery 4; $p<0.01$).

	Operation		P values
	Open (N=27)	**Laparoscopic (N=26)**	
Median age (years; range)	70 (54-84)	71 (38-85)	P=0.96
Sex (Male:Female)	18:9	15:11	P=0.47
Body mass index (kg/m^2, ±SEM)	26.7±1.3	25.9±0.8	P=0.59
Charlson comorbidity index			
2	4	5	P=0.45
3	16	17	
4	5	4	
5	2	0	
ASA grade			
I	4	5	P=0.36
II	20	21	
III	2	0	
IV	0	0	
V	0	0	
Stage			
Dukes A	8	7	P=0.97
Dukes B	7	9	
Dukes C	12	10	
Dukes D	0	0	
Mean operation time (Min, ±SEM)	141.6±10.2	182.5±10.83	*P<0.05
Median blood loss (Ml)	<500	<500	P=0.96
Median length of stay (Days; range)	7 (3-41)	4 (2-11)	#P<0.01

Table 3.3 Demographic and operative data

** statistically significant independent T-test, # statistically significant Mann-Whitney U test. SEM- standard error of mean; Min- minutes; Ml- millilitres*

The Clavien-Dindo classification (CDC) is a validated tool used to grade postoperative complications (Dindo, Demartines et al. 2004) (table 3.4). Increasing scores correlate with severity of complications (ie. CDC 0 equates to an uncomplicated recovery whilst CDC 5 represents patient death in hospital).

CDC	Definition
I	Deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic and radiological interventions
II	Requiring pharmacological treatment, blood transfusion or total parenteral nutrition
III A B	Surgical, radiological or endoscopic intervention Local anaesthetic General anaesthetic
IV A IB	Life-threatening complication Single organ failure Multi-organ failure
V	Death

Table 3.4 Clavien-Dindo classification

CDC- Clavien-Dindo classification

Laparoscopic surgery was associated with a greater proportion of patients having uncomplicated recovery (19 of 26 patients) compared with open surgery (12 of 27 patients) (P=0.514 Fishers exact test). Overall, laparoscopic surgery was associated with fewer, less severe complications than open surgery ($p<0.05$) (table 3.5).

Clavien-Dindo classification	Operation		Statistical significance
	Open (N=27)	Laparoscopic (n=26)	
0	12	19	*P<0.05
1	9	6	
2	4	0	
3A	1	0	
3B	1	1	
4A	0	0	
4B	0	0	
5	0	0	

Table 3.5 Postoperative complications

** statistically significant, Mann-Whitney U test*

3.6 DISCUSSION

This study shows no statistical difference between open and laparoscopic colonic surgery groups regarding age, sex, BMI, tumour stage and blood loss. This allows a degree of confidence that differences observed during the study are likely due to surgical technique only. LS was associated with a longer operating time, shorter inpatient stay and fewer complications than open surgery.

Data was collected from each patient on at least five of the eight study days spanning 6 months. One patient died during the course of the study from pneumonia, almost five months after an open colonic resection (Dukes B) complicated by an anastomotic leak (treated with antibiotics). The patient had been discharged 11 days post surgery and did not receive postoperative chemotherapy.

Laparoscopic colonic surgery was associated with a significantly longer operating time (mean difference of 39 minutes), a shorter hospital stay (difference between medians of 3 days) and fewer complications. These findings mirror those of the 2005 Cochrane review which analysed data from 25 randomised control trials (Schwenk and Kehlet 2004). The meta-analysis reported an improved length of stay (by 1.7 days) and fewer postoperative complications despite an operating time on average 42 minutes longer than open colonic surgery (Schwenk and Kehlet 2004).

The discrepancy between length of stay noted for laparoscopic colonic surgery in our study and those of the Cochrane review could be due to improvements in enhanced recovery protocols over the last decade. The majority of studies

included within the 2005 Cochrane review included regular use of epidurals and limited use of spinal anaesthesia, '*transversus abdominis* plane' block and local anaesthetic wound infiltration devices (Schwenk and Kehlet 2004). The review also highlighted a significantly lower blood loss, by 113mls to 31mls total, within the laparoscopic surgical group (Schwenk and Kehlet 2004). Our analysis of blood loss found no difference between groups however our data arises from an estimate made by the Anaesthetist with the minimum value expressed as <500mls blood loss. Any variations between operative techniques may lie within this 0 to 500mls range.

Particular strengths of this study are the low rate of participant drop-out and the relatively high levels of compliance. The use of routine outpatient appointments and the inpatient stay as study assessment days reduced the number of additional visits participants were required to make. By reducing the potential impact upon patient's daily lives may have helped to reduce drop-out rates and increase overall compliance. The preoperative study assessment occurred after patients were seen for routine clinical pre-operative assessment clinics, whilst the first three postoperative study days were incorporated within each patient's inpatient stay. The 4 week and 6 month study days again coincided with routine follow-up outpatient appointments. Our design allowed frequent assessment of study participants with minimal financial implications regarding travel and parking charges combined with minimal impact upon daily activities.

A second important factor regarding participant compliance is that participants were not moved from clinical settings into research environments for assessments. The researcher was able to assess participants and collect data wherever they were in the hospital. Equipment required for quantification of

inflammatory markers, assessment of muscle architecture and functionality were all portable, being specifically designed to fit into a laptop bag. An additional strength was that five different colorectal surgeons performed the operations included for analysis, and each one used the same enhanced recovery protocol in every case. This improves the heterogeneity within our groups and limits potential bias within the recovery phase. None of the patients had a stoma and a similar number of patients (14) undertook postoperative chemotherapy.

It may be argued that a limitation of this study is that there was no randomisation and that the study was not blinded. This is a pragmatic study, and it is widely accepted that laparoscopic surgery should be offered to the majority of patients requiring colonic resection. By assessing the key demographics of our two groups we highlight potential selection bias. Reassuringly no differences were seen in the key demographics of age, sex, BMI, tumour site and staging. Considering the role of blinding with this study, the researcher gathering data from these patients did not have any clinical role in their care and such was unlikely to have biased the study although we appreciated that the clinicians responsible for the patients potentially may bias the study with their own postoperative practices.

3.7 CONCLUSION

Potential sources of preoperative selection bias were addressed within the design of this study with our open and colonic resection groups being evenly matched regarding age, sex, BMI and tumour stage. Laparoscopic colectomy was associated with reduced length of stay and fewer complications despite having a longer operative time which mirrors published data from several multicentre randomised control trials.

Chapter 4

Surgical stress response

4.1 INTRODUCTION

The stress response is a basic survival mechanism which enables us to recover from injury. The overall aim is to increase the amount of energy generating substances at our disposal to overcome physical and metabolic insults.

Concentration of pro-inflammatory cytokines within blood may predict the degree of surgical stress. Measurement of these cytokines is complex, expensive and labour intensive, which limits use in lengthy longitudinal studies. Potentially white cell counts offer an excellent alternative, being readily available, at low cost within a short time-frame. Recent studies have shown that white cell counts may highlight the severity of surgical stress responses with better accuracy than either serum cytokine (IL-6) concentrations or CRP levels (Yamamoto, Fujita et al. 2004; Sarbinowski, Arvidsson et al. 2005; Servis, Busic et al. 2008; Tabuchi, Shimazaki et al. 2011).

Quantitatively, as well as qualitatively, individual leukocyte sub-type levels vary in response to biochemical mediators and stress hormones (Tabuchi, Shimazaki et al. 2011). Improvement of clinical status following surgery coincides with a gradual increase in lymphocyte counts and concomitant decrease in neutrophil counts (Zahorec 2001). *Tabuchi et al* have recently shown that granulocyte lymphocyte ratio (GLR) acts as an excellent surrogate maker for IL-6 levels in patients having colorectal surgery (Tabuchi, Shimazaki et al. 2011).

Regular assessment of GLR during the postoperative period potentially allows accurate mapping of the SSR throughout the recovery period.

4.2 AIM

1. To validate the use of GLR as a surrogate for IL-6 in the post colectomy period.

2. To compare the postoperative surgical stress response during recovery of patients undergoing open and laparoscopic surgery for potentially curable colonic adenocarcinoma.

4.3 METHOD

4.3.1 Overview

This part of the study adheres to the protocol (see appendix I) covered by ethical approval from the Nottingham Research Ethics Committee I (NRES reference: 13/EM/0031). Interleukin-6 levels were only taken for a sub-set of patients recruited for the larger study to validate the use of granulocyte lymphocyte ratio.

4.3.2 Human interleukin-6 immunoassay

Sample collection

Blood samples for were taken at each study day for a sub-set of patients within the study (n=8). Five millilitres of blood was extracted from each patient and stored within an ethylenediaminetetraacetic acid (EDTA) primed vacutainer (Becton, Dickinson & Co., Plymouth, UK). Each sample was immediately placed in ice and transferred to the laboratory. Serum extraction required each sample to be centrifuged at 3200 revolutions per minute at 4°C for 20 minutes (IEC Centra CL3R, Geneva, Switzerland) and the supernatant decanted using 1 millilitre pipettes (Eppendorf, Hamburg, Germany) into 1.5 millilitre vials for

storage (Eppendorf, Hamburg, Germany) at -80°C (New Bunswick Ultra U570, Eppendorf, Hamburg, Germany).

Sample preparation

Serum samples were left at room temperature to thaw. Thawed serum samples were centrifuged at 3200 revolutions per minute (IEC Centra CL3R, Geneva, Switzerland) for 5 minutes at room temperature. The supernatant was aspirated using sterile pipettes into fresh 1.5 millilitre vials (Eppendorf, Hamburg, Germany).

Immunoassay

A Quantikine® Human IL-6 immunoassay (ELISA) kit (R&D Systems, Minneapolis, MN, USA) was used to calculate IL-6 concentrations within each serum sample. This assay employed a quantitative sandwich enzyme immunoassay technique (Systems 2014). Sterile equipment was used throughout. Reagents were stored at between 2-8°C until needed and warmed to room temperature prior to use. The immunoassay plate had been pre-coated with a monoclonal antibody specific for human IL-6. One hundred micro-litres of Assay Diluent RD1W were pipetted into each well.

The first two columns on the microplate were reserved for controls and calibrations. Positive control was standard human IL-6 (333pg/ml) and negative control consisted of Calibrator Diluent RD6F. A series dilution of the standard human IL-6 with calibrator diluent produced six calibration samples (100pg/ml, 50pg/ml, 25pg/ml 12.5pg/ml, 6.25pg/ml and 3.13pg/ml). The positive and

negative controls together with the six calibration samples were placed into the first column and duplicated for the second column. One hundred micro-litres of serum sample were placed into each well except wells assigned for standards or controls. The entire plate was covered with clear film and left to incubate for 2 hours at room temperature.

A wash buffer was created by adding 20 millilitres Wash Buffer Concentrate to 480 millilitres of distilled water. The mixture was well shaken. Each well was aspirated dry and washed with 400 micro-litres wash buffer. This process was repeated four times. After the last wash and aspiration each plate was blotted dry. Two hundred micro-litres of Human IL-6 Conjugate were pipetted into each well left to incubate for a further 2 hours at room temperature.

Each well was aspirated dry and then washed with wash buffer. The wash buffer was aspirated and each well re-washed four times. Each plate was then blotted dry once more. A substrate solution was created by mixing 12 millllilitres Color Reagent A to 12 millilitres Color Reagent B. The resulting substrate solution (200 micro-litres) was added to each well and left to incubate at room temperature for 20 minutes in the dark. Fifty micro-litres Stop Solution was added to each well and the plate gently shaken for 1 minute until a blue-yellow colour change was seen. A microplate reader (Multiskan Ascent, Thermo Fisher Scientific, Waltham, Mass, USA) was used to determine the optical density of each well. The reader was set to 450 nanometers with wavelength correction set to 540 nanometers.

Creation of standard curves for calibration

All duplicate readings were averaged and subtracted from the zero standard optical density reading. A standard curve was created by plotting the mean absorbance for each control and calibration sample on the y-axis against the known concentration on the x-axis and a line of best fit was created (figure 4.1). Following manufactures instructions, a Pearson product-moment correlation coefficient was produced for the values used in the standard curve (r=0.996). The p value for this correlation coefficient is p<0.01.

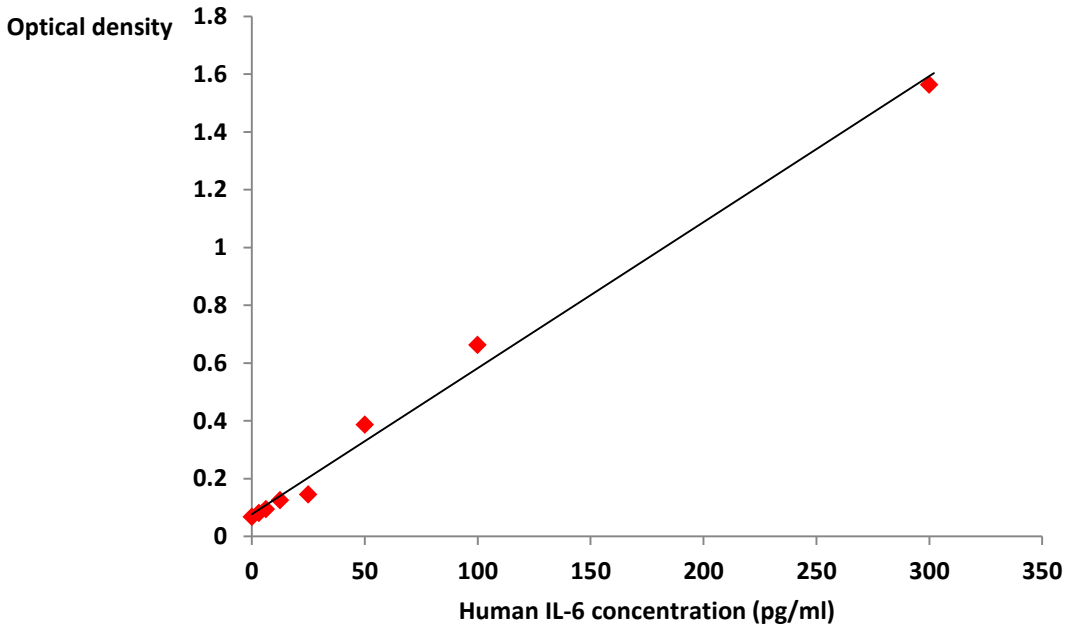


Figure 4.1 Standard curve for human IL-6 immunoassay calibration

The red dots highlight the mean values obtained for the controls (positive and negative) and calibration solutions (100pg/ml, 50pg/ml, 25pg/ml, 12.5pg/ml, 6.25pg/ml 3.13pg/ml). The solid black line represents the line of best fit (r=0.096; p<0.01).

Serum IL-6 concentration calculation

Individual values for IL-6 concentration were produced for each well by equating the optical density value obtained to a predicted IL-6 concentration using the stand curve data created above. Each participant sample was run in duplicate therefore mean IL-6 concentrations are presented.

4.3.3 Granulocyte lymphocyte ratio

Blood samples for GLR were taken on each study day (see figure 3.1) for all patients within the study (n=53). Five millilitres of blood was extracted and stored within an EDTA primed vacutainer (Becton, Dickinson & Co., Plymouth, UK). This vial was then transported to the Pathology Laboratory, Department of Pathology, Royal Derby Hospitals NHS Trusts for analysis where differential white cell counts were obtained using an automated multi-plate analyser (Caretium Medical Instruments Co., Shenzen, China). Results obtained included neutrophil, eosinophil, basophil and lymphocyte counts. The neutrophil, eosinophil and basophil counts were combined giving an overall granulocyte count. To produce a value for GLR the overall granulocyte count was divided by the lymphocyte count.

4.4 RESULTS

4.4.1 Interleukin-6 concentration

Two of the eight patients entered into the validation study were excluded from final analysis. One patient suffered postoperative bleeding and returned to theatre for emergency surgery. This patient was transfused 3 units of packed red cells. The second patient received 2 units packed red cells postoperatively due to symptomatic shortness of breath having been known to be anaemic preoperatively.

Data for mean serum IL-6 concentration during the postoperative period for the remaining 6 patients is shown in figure 4.2. Preoperative mean IL-6 concentration was 3.57±1.60pg/ml. Postoperative mean IL-6 concentrations were 123.8±55.37pg/ml on day 1, 56.7±25.13pg/ml on day 3, 10.25±5.10pg/ml on day 14, 7.19±2.09pg/ml on day 28 and 3.19±1.53pg/ml on day 42.

The 6 eligible patients each had 6 time points measured. This gave 36 potential IL-6 values which could be correlated with granulocyte lymphocyte data. Figure 4.3 depicts the correlation between each individuals IL-6 serum concentration and granulocyte lymphocyte ratio ($r=0.66$, $p<0.001$).

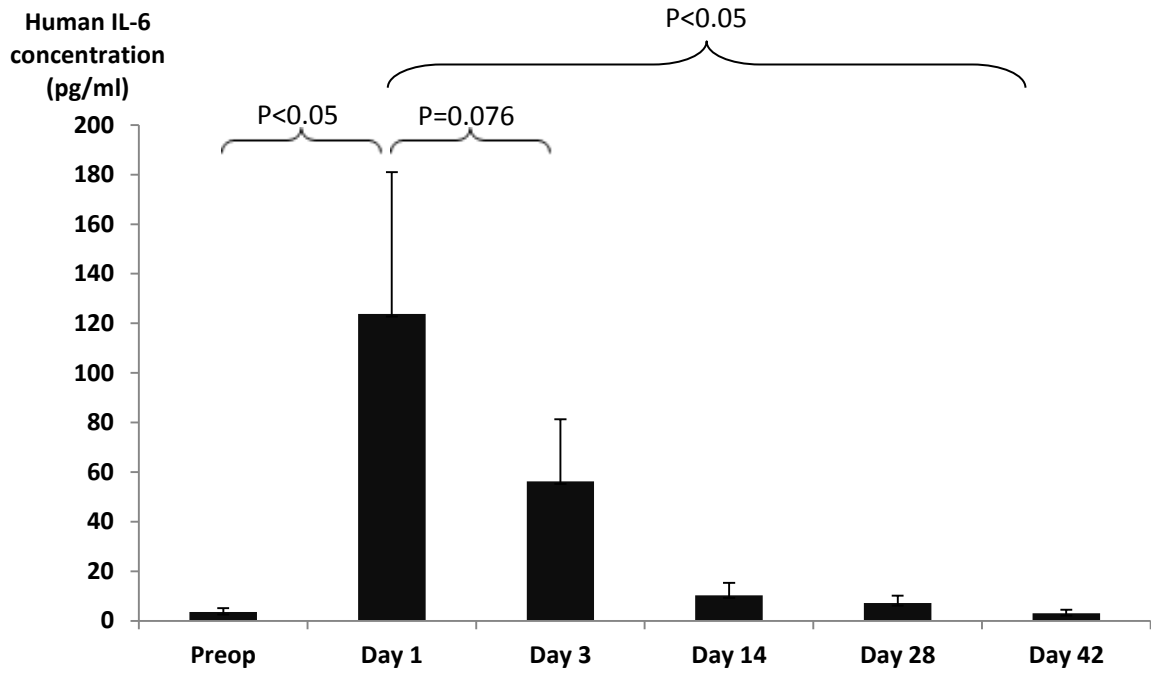


Figure 4.2 Human IL-6 concentrations post colectomy.

The data depicted is mean IL-6 concentrations from eligible patients (n=6). Error bars represent standard error of the mean. Statistically significant differences are marked (paired t-test).

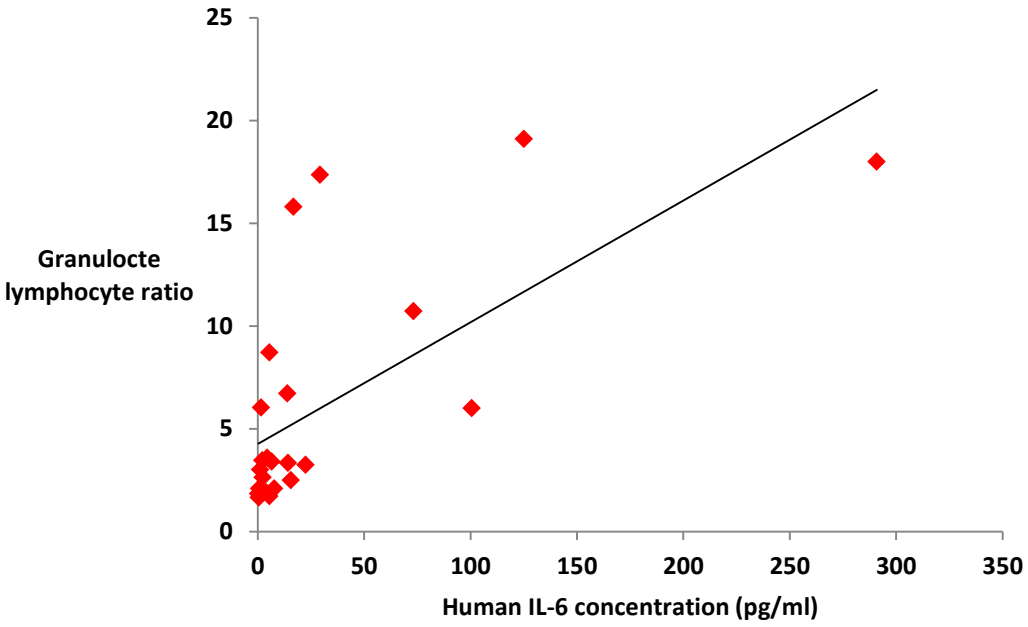


Figure 4.3 Correlation between IL-6 and granulocyte lymphocyte ratio

Each red dot represents a sample retrieved from one participant. The solid black line represents the line of best fit (r=0.66, p<0.001).

4.4.2 Granulocyte, lymphocyte & granulocyte lymphocyte ratio

Data collected for granulocyte counts, lymphocyte counts and granulocyte lymphocyte ratio is shown in figures 4.4, 4.5 and 4.6. No statistical differences were noted between open (n=27) and laparoscopic (n=26) colonic surgery on any study day for granulocyte count, lymphocyte count and granulocyte lymphocyte ratio.

Mean preoperative granulocyte counts were 5.54±0.39 $x10^9$/L and 5.24±0.36 $x10^9$/L for open and laparoscopic patients respectively. This rose to a peak value of 10.18±0.59 $x10^9$/L for the open group and 10.2±0.58 $x10^9$/L for the

laparoscopic group at day 1. After day 1 granulocyte count dropped to 8.47±0.58 x10^9/L for the open group and 7.83±0.69 x 10^9/L for the laparoscopic group. Granulocyte count dropped steadily for both groups until the 6 months recording where levels were found to be below preoperative values ($p<0.01$).

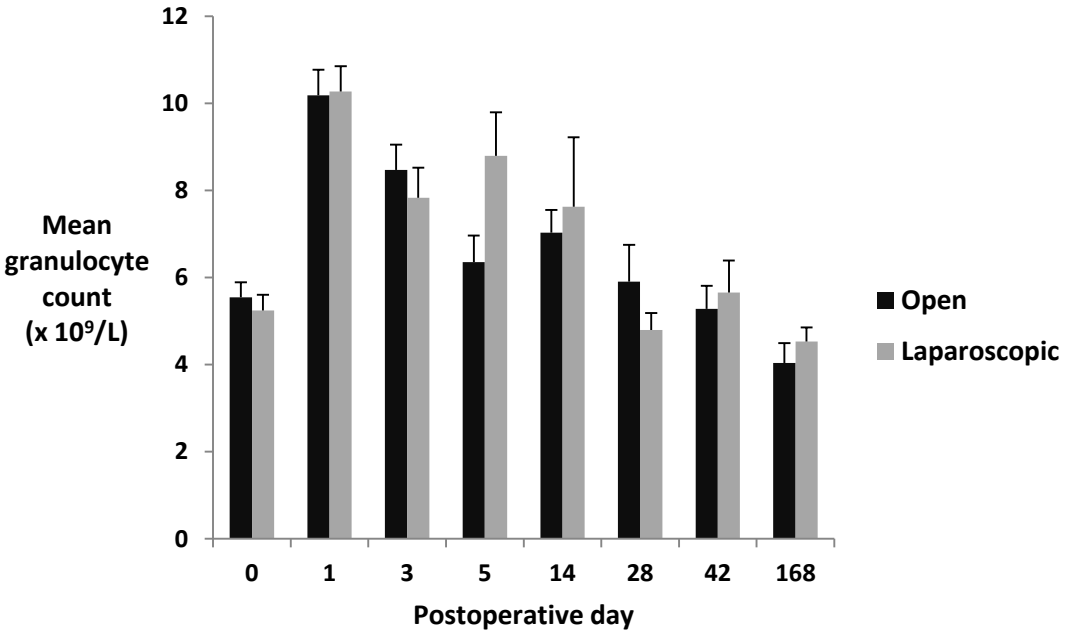


Figure 4.4 Postoperative granulocyte count

Error bars highlight standard error of the mean. No statistical difference between groups noted.

Preoperative lymphocyte count was 1.76±0.1 x10^9/L and 1.81±0.13 x10^9/L for open and laparoscopic surgery groups respectively. These dropped sharply post surgery being at their lowest level on day 3 (open surgery 1.10±0.12 x10^9/L, laparoscopic surgery 1.17±0.11 x 10^9/L). From here levels rose steadily reaching preoperative values at day 42.

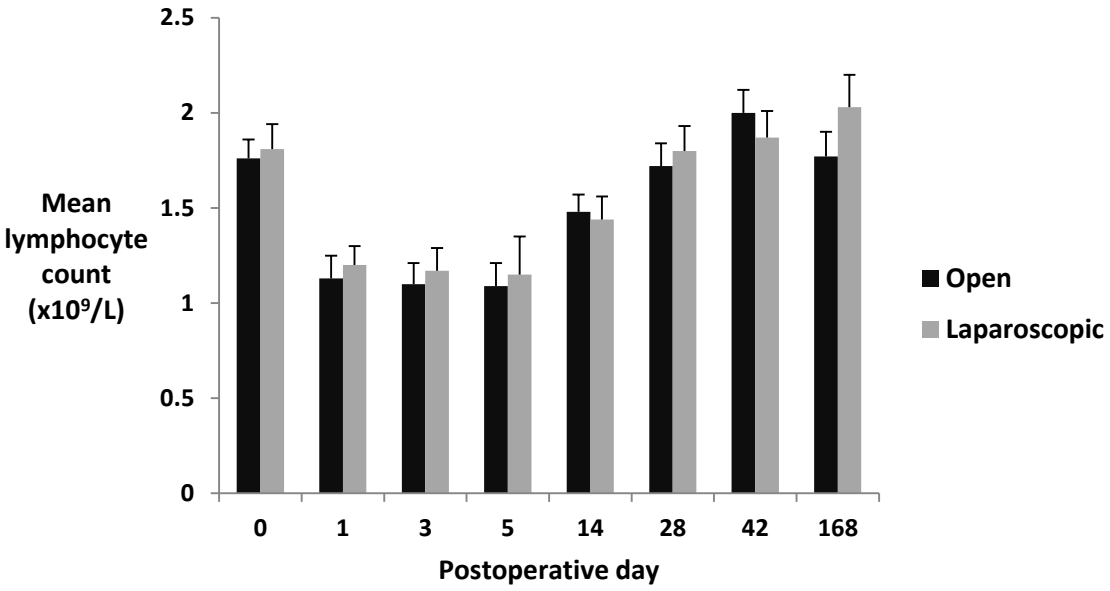


Figure 4.5 Postoperative lymphocyte count

Error bars highlight standard error of the mean. No statistical differences between groups

Results for granulocyte lymphocyte ratio are shown in figure 4.6. There were no statistical differences between open and laparoscopic colonic surgery groups on any day (independent T-tests). Preoperative GLR was 3.07±0.18 for the open surgery group and 2.84±0.32 for the laparoscopic surgery group. GLR rose post surgery peaking at day 1 (11.19±1.1 and 9.77±1.2 respectively). After this GLR decreased steadily and at 6 months was found to be lower than preoperative values (2.18±0.2 and 2.00±0.26 respectively, $p<0.01$). Both open and laparoscopic colonic surgery groups noted statistical differences between several study days (paired T-tests).

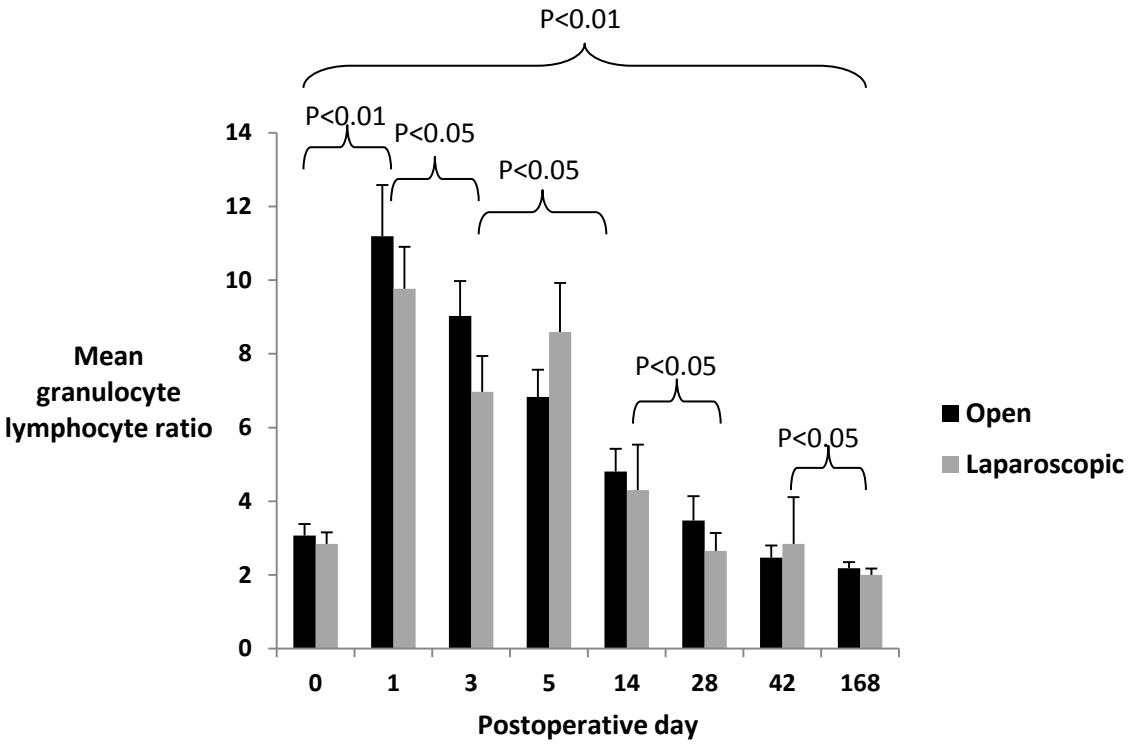


Figure 4.6 Mean postoperative granulocyte lymphocyte ratio

Error bars highlight standard error of the mean. Statistically significant differences between study days are shown (paired t test).

4.5 DISCUSSION

This study attempts to validate the use of granulocyte lymphocyte ratio as a surrogate marker for IL-6 post colectomy within our institution. GLR was used to assess variations in between open and laparoscopic colectomy patients in the postoperative period.

IL-6 concentration was elevated within our subset population after colonic resection. Peak IL-6 concentration occurred on the first postoperative day and levels decayed steadily over the following 6 weeks. No differences in IL-6 concentrations were detected between open and laparoscopic surgery. This component of the SMMAC study was not powered to detect serum IL-6 differences between our two groups. Our aim was to validate the correlation between IL-6 and GLR within our institution. Participant IL-6 concentrations correlated well with granulocyte lymphocyte ratio despite a small sample size. Granulocyte lymphocyte ratio may be a surrogate maker for IL-6 levels in postoperative colectomy patients.

Several randomised controlled trials have presented statistically significant differences in serum IL-6 levels between patients undergoing open and laparoscopic colonic surgery (Harmon, Senagore et al. 1994; Delgado, Lacy et al. 2001; Pascual, Alonso et al. 2011). These studies note that serum IL-6 concentrations peak within the first few hours after surgery (maximum 4 hours) and then drop sharply over the next 3 days (Delgado, Lacy et al. 2001; Pascual, Alonso et al. 2011). During this peak there is a detectable difference in serum IL-6 concentrations between the groups. Clinical researchers believe this observation may explain, at least in part, the short-term benefits of minimally invasive surgery compared with open colectomy (Delgado, Lacy et al. 2001; Guillou, Quirke et al. 2005). Our study did not find any significant differences in

serum IL-6 levels probably due to the small sample size and the time-points chosen for measurement. Our first postoperative measurement was at 24 hours after surgery which almost certainly missed peak serum IL-6 levels where significant differences between the groups may exist.

It is assumed potential differences in IL-6 levels are related to the laparotomy and not the surgical dissection, which is the same, independent of technique used. Studies comparing peritoneal IL-6 levels with serum concentrations in patients undergoing either open or laparoscopic colectomy have noted IL-6 levels are disproportionally higher within the peritoneal fluid (300-fold) than the serum, confirming that local inflammatory responses are much greater than systemic ones (Wu, Sietses et al. 2003). No differences were noted in peritoneal IL-6 levels between the two operative groups, indicating that both surgical approaches may cause the same intra-abdominal trauma, however elevated levels of serum IL-6 immediately after open surgery may indicate that the laparotomy itself and not necessarily surgical dissection is responsible for these differences.

Our results mirror those of *Tabuchi et al* who reported a significant correlation ($r=0.74$, $p<0.001$) between granulocyte lymphocyte ratio and IL-6 levels (Tabuchi, Shimazaki et al. 2011). *Tabuchi et al* assessed human IL-6 concentrations and GLR at 5 time points (preoperatively, postoperative day 1, day 3 and day 7) in patients undergoing colorectal surgery (n=104). They too noted wide variation in individual IL-6 concentrations. A possible explanation may be that whilst a SSR occurs in every individual the extent of the SSR is not uniform. Our study and that of *Tabuchi et al*, used identical methods to assess IL-6 concentrations and the GLR. Although our sample size is small (n=36) the

similarity between our results and those of *Tabuchi et al* validates the hypothesis that GLR may be used a surrogate for serum IL-6 levels in colectomy patients. This confirmation enables us to review the inflammatory changes associated with the surgical stress response with greater regularity during an extended postoperative period within a large population.

There were no statistical differences in granulocyte or lymphocyte counts and the granulocyte lymphocyte ratio between patients having open and laparoscopic colonic surgery at any time point over the six month study period. The concentration of granulocytes within the serum significantly increased after surgery peaking on the first postoperative day and dropped gradually over the next 28 days. Between days 28 and 42 levels plateau until the 6 month study day, where granulocyte counts are significantly lower than those noted preoperatively. Lymphocyte counts follow an inverse trend. Lymphocyte concentrations decreased significantly on the first postoperative day and remained at this level until day 5. After day 5 levels increase gradually, becoming equivalent to preoperative values and at day 28 and by day 32 (6 weeks postoperatively) levels were significantly elevated compared to preoperative values. Values for granulocyte lymphocyte ratio peaked on the first postoperative day and drop gradually over the following 6 weeks. At six months serum granulocyte levels are significantly below preoperative values.

To understand these trends we must consider the mechanisms behind the stimulation and release of granulocytes and lymphocytes. Elevated IL-6 levels associated with an increased granulocyte and decreased lymphocyte count have been noted previously in patients with colorectal cancer (Shimazaki, Tabuchi et al. 2015).

Preoperative elevation of IL-6 and granulocyte counts may be explained by the invasive nature of colon cancer. Progression and proliferation of colonic cancer cells causes localized tissue haemorrhage, ischaemia and necrosis which stimulates a variety of immune and inflammatory responses (Shimazaki, Tabuchi et al. 2015). These responses include an elevated granulocyte count and the immunosuppression of lymphocytes which are controlled by the cytokine network (IL-6, IL-1 and tumour necrosis factor) (Malicki, Winiarski et al. 2009). The most important cytokine, IL-6 stimulates the production of acute-phase proteins and promotes the proliferation of immature neutrophils within the circulation. Preoperative IL-6 levels are associated with the extent of local tumour invasion, progression and subsequent prognosis of colorectal cancers (Belluco, Nitti et al. 2000; Galizia, Orditura et al. 2002).

Postoperative elevations in serum IL-6 are associated with tissue injury, making IL-6 a marker for surgical stress. In line with these assertions, serum IL-6 concentrations are high in the early postoperative phase and decreased gradually over time as tissues undergo repair. As IL-6 levels decrease there is less stimulus for granulocyte recruitment and thus the granulocyte count steadily declines whilst the lymphocyte count rises.

As stated previously measurement of IL-6 is time-consuming and expensive. This has limited researchers to viewing only a few time points in the early postoperative period during longitudinal studies. Granulocyte lymphocyte ratio enables us to uniquely extend the time-frame for analysis to a period of 6 months. We have shown by 6 weeks post surgery granulocyte lymphocyte ratio returns to preoperative levels, and continue's to decrease slowly with time. At 6 months granulocyte lymphocyte ratio decreases further, to a level significantly

below preoperative values. This may be due to the absence of inflammation driven by the local invasiveness of cancer after removal of the tumour.

Use of granulocyte lymphocyte ratio has increased over the last year with researchers publishing material correlating elevated granulocyte lymphocyte ratios prior to surgery with poorer overall prognoses for patients with colorectal cancer. A study by *Shimazaki et al* goes further by suggesting elevated granulocyte lymphocyte ratio prior to surgery may be an independent risk factor for postoperative complications, in particular intraoperative haemorrhage (Tabuchi, Shimazaki et al. 2011; Shimazaki, Goto et al. 2013; Shimazaki, Tabuchi et al. 2015).

4.6 CONCLUSION

Use of granulocyte lymphocyte ratio, a potential surrogate for IL-6 enables assessment of the surgical stress response in the postoperative period. The surgical stress response may continue for up to 4 weeks postoperatively but after six months inflammatory levels fall below preoperative values.

Chapter 5

Skeletal muscle architecture and muscle protein synthesis

5.1 INTRODUCTION

Open colorectal surgery is associated with an overall loss of skeletal muscle mass resulting in reduced physical function and increased fatigue 1 month after surgery (68-72) (Jensen, Houborg et al. 2011). Ongoing skeletal muscle modulation over the following weeks may continue to impact upon skeletal muscle mass and reduce physical function further.

To quantify changes in skeletal muscle mass during recovery, regular repeated measurements are required. Traditionally researchers have used three different techniques: bioimpedance analysis, CT and DXA (Jensen, Houborg et al. 2011). A combination of radiation exposure, expense and the need for specialist equipment limit regular use of CT and DXA, whilst extrapolation of data obtained from bioimpedance analysis is controversial in the immediate postoperative period (Takai, Ohta et al. 2013; Abe, Loenneke et al. 2015).

B-mode ultrasound reliably measures variations of skeletal muscle architecture within longitudinal observational healthy volunteer studies, and these variations are known to correlate well with fluctuations in muscle mass (Abe, Kawakami et al. 1997).

Isotope-labelled, non-radioactive amino acids have allowed us to quantify the changes in whole-body protein synthesis and breakdown after surgery however this technique is limited to recording data over a short time frame. Refinements in deuterium bio-labelling techniques may allow accurate measurements of muscle protein synthesis rates over several weeks. In order to fully assess skeletal muscle modulation after colorectal surgery we need to observe changes

within skeletal muscle safely, on a regular basis over a longer period of time and include patients undergoing laparoscopic procedures. Measurement of variations in skeletal muscle architecture with B-mode ultrasound and quantification of muscle protein synthesis rates with deuterium labelling techniques have the potential to comprehensively quantify changes that occur after colorectal cancer surgery.

5.2 AIM

1. To use B-mode ultrasound to compare changes in muscle architecture after open and laparoscopic surgery for colorectal adenocarcinoma.

2. To use deuterium oxide (D_2O) as a clinical bio-label to assess muscle protein synthesis rates over a period of 42 days, commencing prior to colonic cancer surgery and continuing for 6 weeks.

5.3 METHOD

5.3.1 Overview

Muscle architecture assessments were attempted in every participant. Assessment of muscle protein synthesis rates was limited to a sub-set population of 8 patients due to technical problems associated with the mass spectrometer and that this was a feasibility study.

5.3.2 Skeletal muscle architecture

5.3.2.1 Image capture

A portable LogicScan 128 INT-1Z KIT B-mode ultrasound scanner (Telemed UAB, Vilinius, Lithuania) with a 50 millimetre 9-3MHz ultrasound probe was used to generate every image. Recruited patients had images taken on each study day. Each patient rested for 5 minutes prior to commencing any measurements.

An easily reproducible point, 60% along the axis from the greater trochanter of the femur and the knee joint-line was marked on the right lower limb. At this point a sagittal image was captured to delineate the anterior and posterior borders of the *vastus lateralis* (VL) muscle.

Using a tape measure, a second mark was placed at the midpoint between the anterior and posterior aspect of this muscle. This mark is referred to as the point of measurement (POM) (figure 5.1). A coronal image was captured of the muscle at this point and later used for assessment of fascicle length, muscle thickness and pennation angle (figure 5.2).

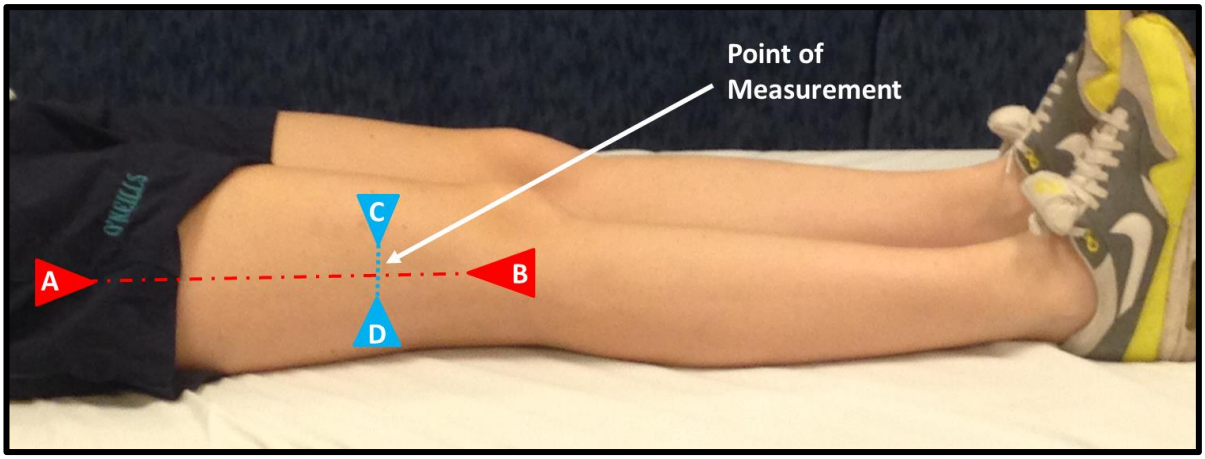


Figure 5.1 Marking point of measurement of right *vastus lateralis*

An imaginary line (red) is marked from the greater trochanter (A) to the lateral condyle of the femur (B). At a point 60% down its length ultasonography is used to delineate the (C) superior and inferior borders (D) of the vastus lateralis in the coronal plane (blue). The point of measurement lies midway between these points.

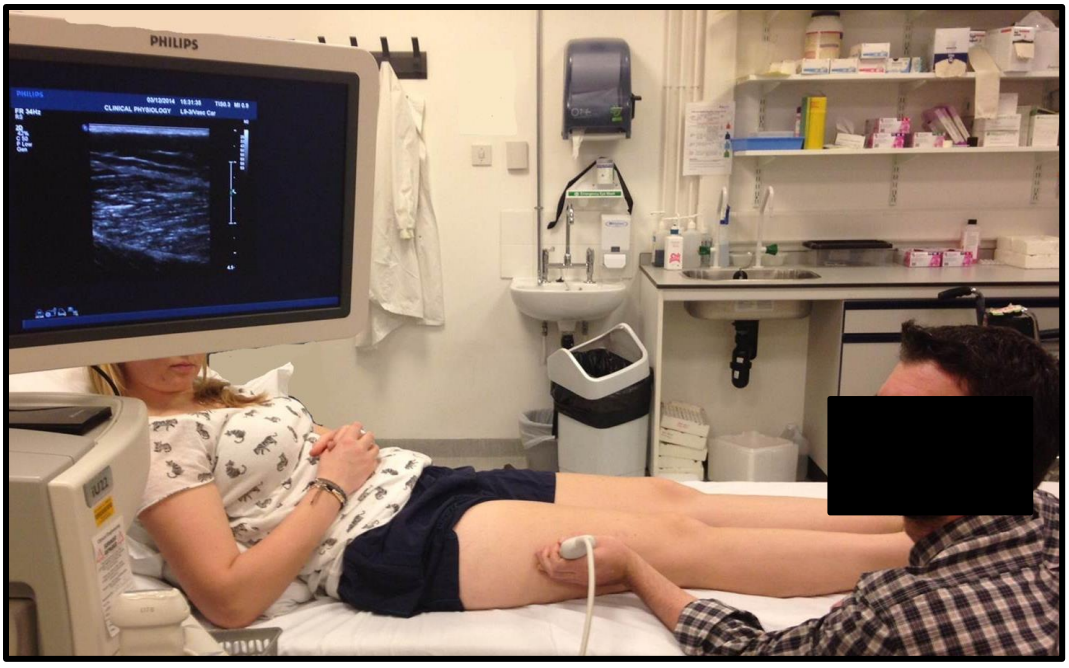


Figure 5.2 Image capture of *vastus lateralis* at the POM

A researcher using ultrasonography to capture an image of the right vastus lateralis muscle at the point of measurement.

5.3.2.2 Analysis

Analysis was undertaken using ImageJ™ software (National Institutes of Health, Bethesda, USA). Muscle thickness was measured as the perpendicular distance between superficial and deep aponeuroses of VL at the POM. Pennation angle was calculated as the mean of the angles measured between the long axis of 3 fascicles and the deep aponeurosis. Fascicle length was measured as the length of a straight line orientated parallel to the calculated pennation angle, centred on the POM (figure 5.3). In some instances, a small portion of the fascicle extended outside the ultrasound window and it was necessary to estimate this non-visible portion using a linear extrapolation of fibres and aponeuroses (figure 5.4) (Erskine, Jones et al. 2009).

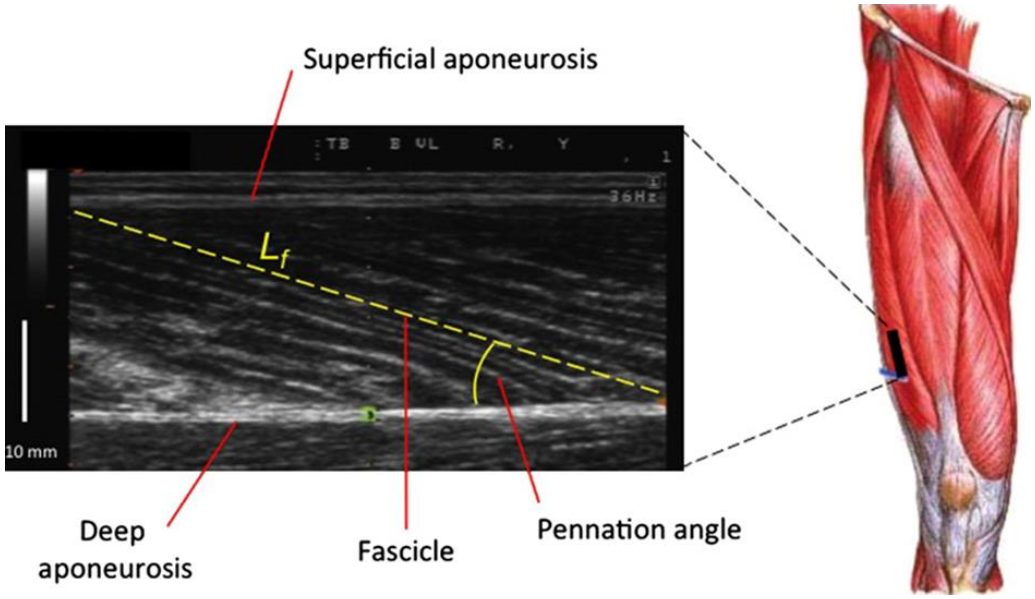


Figure 5.3 Fascicle length at the point of measurement of the right *vastus lateralis*

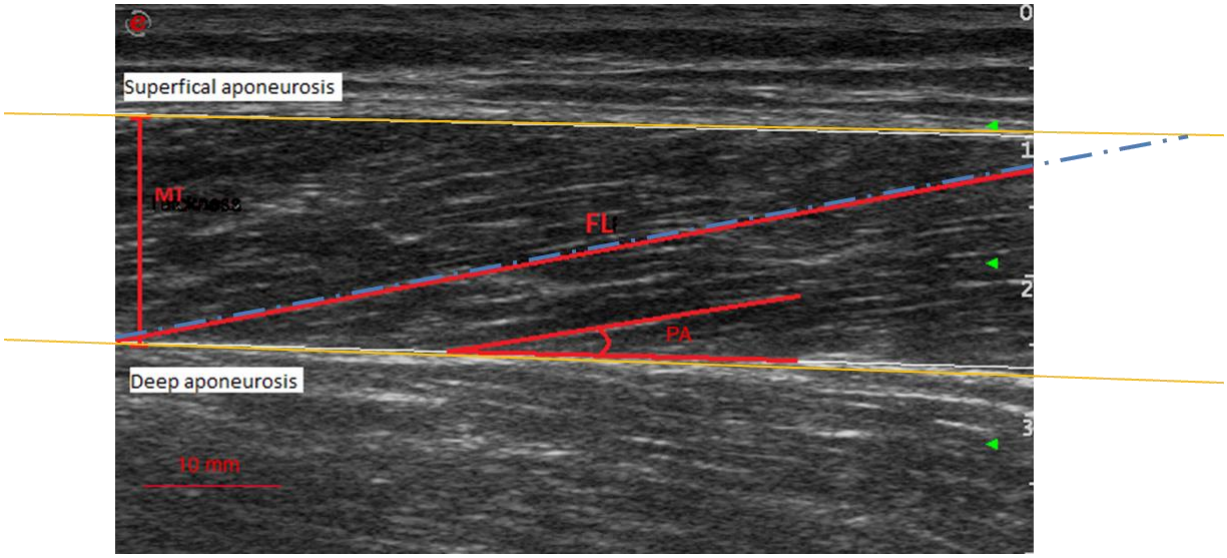


Figure 5.4 Snapshot of an image captured for muscle architecture analysis. *Yellow lines depict extrapolated aponeuroses and the broken blue line depicts an extrapolation of fascicle length allowing accurate measurement. MT- muscle thickness; FL- fascicle length; PA- pennation angle*

5.3.3 Muscle protein synthesis

5.3.3.1 Study procedures

Muscle protein synthesis rates were calculated for a sub-set of patients recruited to the study (n=8). Each patient was given 3 millilitres/kilogram of deuterium oxide (D_2O) (70 atom%; Sigma-Aldrich, Poole, UK) on study day 1, 14 and 28. Two muscle biopsies were taken 6 weeks apart. The first, whilst the patient was anaesthetised prior to the operation commencing and the second, under local anaesthetic (10mls 1% lidocaine) 6 weeks postoperatively (as part of the study day 42 assessment). All muscle biopsies were taken from *vastus lateralis* (mid-thigh) under sterile conditions, using the conchotome biopsy technique (B. Braun Medical Ltd, Sheffield, UK) (Dietrichson, Coakley et al. 1987). Each

specimen was rapidly dissected free of fat and connective tissue, washed in ice-cold saline, and then frozen in liquid nitrogen and stored at −80°C until further analysis. Single venous blood samples were collected on each study day and during the operation. The blood was stored in lithium heparin-coated vacutainers (Beckton, Dickson and Co. Plymouth, UK); these were immediately cold centrifuged at 3200 revolutions per minute, the plasma fraction aliquoted and then frozen at −80°C until analysis. A schematic of the D_2O bio-labelling phase of the study is provided in figure 5.5.

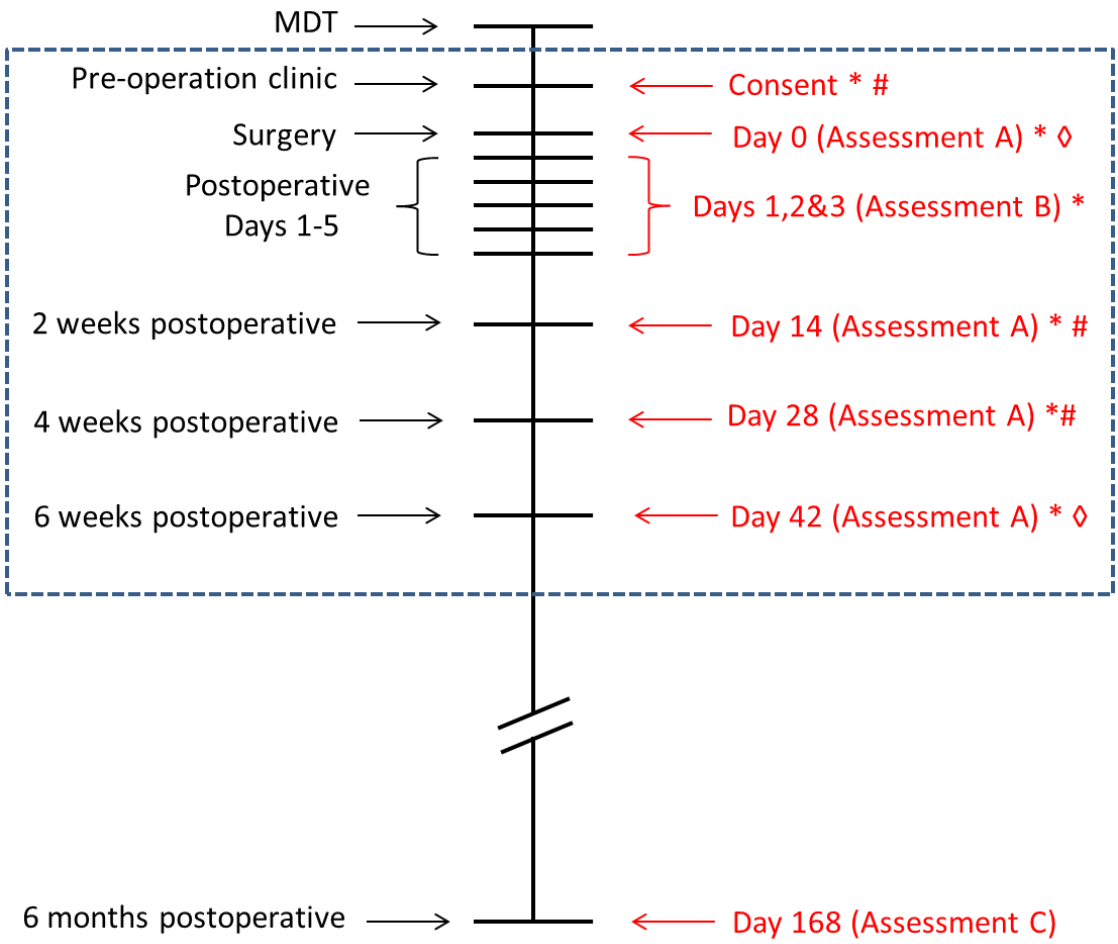


Figure 5.5 Analysis of muscle protein synthesis within SMMAC study.

*The boxed area indicates the 6 week sub-set study for muscle protein synthesis rates. The highlighted area shows additional assessments for these patients. * blood tests; # D20 administered; ◇ muscle biopsy*

5.3.3.2 Body water enrichment

Body water enrichment was determined by aliqouting 50-100 micro-millilitres of plasma in the cap of an inverted autosampler vial and placing this upon a heating block set at 90°C for 2 hours.

Water distillate was collected by rapidly cooling each vial on ice for 10 minutes and transferring the sample into a fresh vial. 1 micro-millilitre of liquid sample was injected directly injection into a high-temperature conversion elemental analyzer (Thermo Finnigan, Thermo Scientific, Hemel Hempstead, UK) connected to an isotope ratio mass spectrometer (IRMS; Delta V Advantage, Thermo). To minimize the effect of carryover between samples, each was injected a minimum of four times.

To validate the accuracy of the high-temperature conversion elemental analyzer for measuring body water enrichment the analysis was repeated with two participants plasma sets using GC-Pyrolysis-IRMS (Trace GC isolink Delta V Advantage; Thermo Scientific) and a modification of the protocol by *Mahsut et al.* (Mahsut, Wang et al. 2011). 100 micro-litres of each sample was incubated with 2 micro-litre of 10 M NaOH and 1 µl of acetone for 24 hours at room temperature; this high pH incubation leads to the exchange of deuterium from water with the hydrogen positions on the acetone. Following incubation the acetone was extracted into 200 micro-litres of n-heptane, and 0.5 micro-litres of the heptane phase was injected into the GC-Pyrolysis-IRMS for analysis. A standard curve of known D_2O enrichment was run alongside the samples for calculation of enrichment.

5.3.3.3 Isolation and derivatization of muscle protein synthesis rates

For isolation of myofibrillar fractions, ~30–50 mg of muscle was used. The muscle was homogenized in ice-cold homogenization buffer [50 mM Tris·HCl (pH 7.4), 50 mM NaF, 10 mM β-glycerophosphate disodium salt, 1 mM EDTA, 1 mM EGTA, and 1 mM activated Na3VO4 (all from Sigma-Aldrich, St Louis, MO, USA)] and a complete protease inhibitor cocktail tablet (Roche, West Sussex, UK) at 10 µl/µg tissue. Homogenates were rotated for 10 min, and the supernatant was collected by centrifugation at 13,000 rpm for 5 min at 4°C.

The myofibrillar pellet was solubilized in 0.3 M NaOH and separated from the insoluble collagen by centrifugation, and the myofibrillar protein was precipitated with 1 M perchloric acid. The sarcoplasmic proteins were precipitated from the initial homogenate supernatant fraction with 1 M perchloric acid and separated by centrifugation. The insoluble collagen fraction was washed sequentially with 0.3 M NaOH, 70% ethanol, 0.5 M acetic acid, and 0.5 M acetic acid and isolated by centrifugation. Protein-bound AAs from myofibrillar were released using acid hydrolysis by incubating in 0.1 M HCl in Dowex H^+ resin slurry overnight before being eluted from the resin with 2 M NH4OH and evaporated to dryness. The AAs were then derivatized as their n-methoxycarbonyl methyl esters (MCME) according to the protocol of *Husek et al* with slight modification (Husek and Liebich 1994).

The dried samples were re-suspended in 60 µl of distilled water and 32 µl of methanol, and following a brief vortex, 10 µl of pyridine and 8 µl of methylchloroformate were added. Samples were vortexed for 30 seconds and left to react at room temperature for 5 min. The newly formed MCME AAs were then extracted into 100 µl of chloroform; any remaining water was removed

from the sample with the addition of a molecular sieve. Incorporation of deuterium into protein bound alanine was determined by gas chromatography-pyrolysis-isotope ratio mass spectrometry (Delta V Advantage; Thermo Scientific) alongside a standard curve of known l-Alanine-2,3,3,3-d4 enrichment.

5.3.3.4 Plasma alanine enrichment

Plasma (200 µl) proteins were precipitated with 100% ethanol, the supernatant evaporated to dryness and was reconstituted in 0.5 M HCl, and the lipid fraction was removed using ethyl acetate extraction; alanine was then converted to its MCME derivative, as described above. All samples were run alongside an l-alanine-2,3,3,3-d4 standard curve. Enrichment of alanine was then determined using gas chromatography-mass spectrometry (GC-MS; MD800, Fison, UK) and single ion monitoring of m/z 102, 103, 104, 105, and 106.

5.3.3.5 GC-pyrolysis IRMS deuterium analyses.

Prior to each analysis, the IRMS system was calibrated and tested for measurement accuracy and was not used unless it fell within the manufacturer's technical specifications. For GC-pyrolysis-IRMS analysis of acetone and MCME alanine samples were separated on a DB-wax column (30 m × 0.32 mm × 0.25 µm; Agilent J & W) following split-less injection. The oven temperature program for acetone was set at 50°C and held for 2 minutes before being increased at a rate of 30°C/min until a temperature of 240°C was reached. This was then held for 2 min. For alanine the oven temperature program commenced at 70°C and held for 3 minutes before being increased at a rate of 10°C/min to 240°C. Samples were held at this temperature for 15 minutes.

The separated samples were then passed through a high temperature (1,420°C) conversion reactor, where the analytes were converted to ^{2}H gas before being directed to the IRMS, where the $^{2}H/^{1}H$ ratio was determined. For high-temperature conversion elemental analyzer analysis of body water enrichment, we directly injected liquid into the high-temperature conversion elemental analyzer, where samples were immediately converted to H^{2} gas. Sample gases were directed to the IRMS, where the $^{2}H/^{1}H$ ratio was determined. The deuterium isotopic enrichment provided as δ2H was converted to atom% using the following equation:

$$Atom\ \% = \frac{100\ x\ (\delta 2\mathrm{H\ x}\ 0.001 + 1)}{1 + AR\ (\delta 2\mathrm{H\ x}\ 0.001 + 1)}$$

where AR represents the absolute ratio constant for deuterium based on the Vienna standard mean ocean water standard (VSMOW) and equates to 0.00015595.

This was then converted to atom percent excess (APE) by correcting for baseline sample, i.e., background enrichment. If any sample fell outside the dynamic range of the instrument, these were re-injected or re-prepared. In this way values for APE *alanine* (percentage of deuterium bound alanine in muscle) and APE *precursor* (percentage of deuterium bound alanine in plasma) were created.

5.3.3.6 Calculation of Muscle protein synthesis rates.

The rates for muscle protein synthesis were determined from the incorporation of deuterium-labelled alanine into protein, using the enrichment of body water (corrected for the mean number of deuterium moieties incorporated per alanine, 3.7) as the surrogate precursor labelling between subsequent biopsies. The standard equation used is shown below,

$$MPS\ rate = -Log[\frac{\{1-(APE\ alanine/APE\ precursor)\}}{t}]$$

where rate of MPS is presented as %/day, APE *alanine* is the deuterium enrichment of protein-bound alanine muscle, APE *precursor* is mean precursor enrichment over the study and *t* is the time period between muscle biopsies.

5.4 RESULTS

5.4.1 Muscle architecture

5.4.1.1 Muscle thickness, pennation angle and fascicle length

An image was captured for every subject preoperatively and during the first three days postoperatively. The majority of patients returned for follow-up visits on the assigned study days and the data collection rates for these days are reported in chapter 3. Measurements for muscle thickness, fascicle length and pennation angle were calculated from every image captured. Mean values for muscle thickness, pennation angle and fascicle length from both groups are shown in table 5.1.

		Pre-op	2 weeks	4 weeks	6 weeks	6 month
Open	MT (mm±SEM)	20.1 (±0.67)	19.0 (±1.44)	18.1 (±0.92)	17.9 (±1.22)	20.5 (±0.87)
	PA (°±SEM)	12.5 (±0.31)	11.7 (±0.49)	11.2 (±0.37)	10.9 (±0.35)	12.63 (±0.31)
	FL (mm±SEM)	84.2 (2.6)	85.4 (4.2)	87.5 (1.9)	82.7 (4.9)	86.4 (2.9)
Laparoscopic	MT (mm±SEM)	20.5 (±0.74)	19.7 (±0.69)	19.2 (±0.85)	19.6 (±0.84)	20.4 (±0.67)
	PA (°±SEM)	12.6 (±0.34)	11.8 (±0.32)	12.1 (±0.42)	12.3 (±0.39)	12.3 (±0.30)
	FL (mm±SEM)	83.4 (2.9)	86.3 (4.1)	83.4 (1.9)	85.3 (3.6)	87.1 (4.2)

Table 5.1 Mean values for muscle thickness pennation angle and fascicle length *MT- muscle thickness; PA- pennation angle; FL- fascicle length*

The mean muscle thickness of *vastus lateralis* measured at 2 weeks postoperatively decreased within both open and laparoscopic colectomy groups. Although there is a difference between the groups at this time point this was not significant. Mean muscle thickness continues to drop at 4 weeks post surgery, however at this time point there is a significant difference between the groups ($p<0.05$). At 6 weeks, the mean muscle thickness in both groups plateau with a significant difference still noted between the groups. Maximal decrease occurred 6 weeks postoperatively and averaged 8% in the open group. Six months post surgery both groups show a significant increase in muscle thickness to preoperative values.

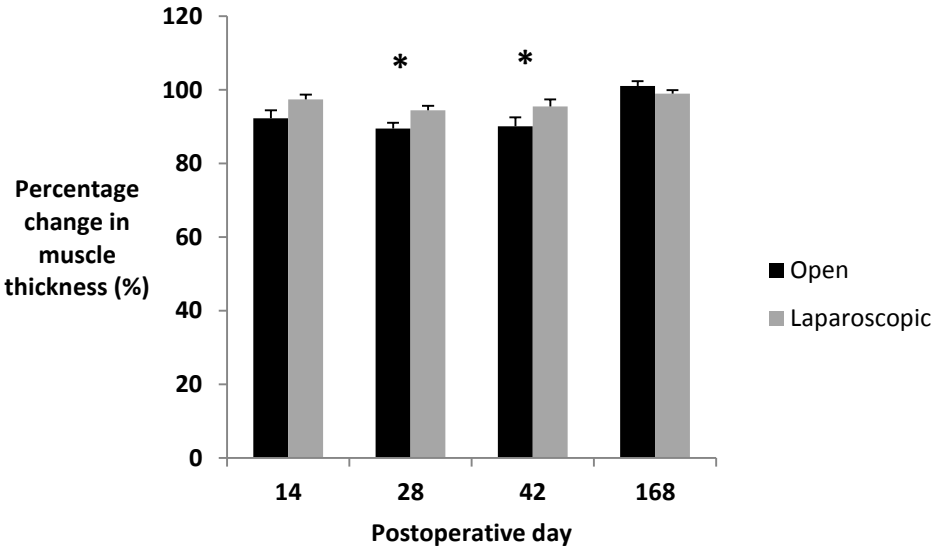


Figure 4.6 Percentage change in muscle thickness

*Preoperative values are used as baseline readings (100%). * refers to a statistically significant difference between groups ($p<0.05$).*

Data for pennation angle is shown in figure 5.7. Pennation angle measurements decreased within the open colectomy group at 2, 4 and 6 weeks post surgery before increasing to preoperative levels at 6 months. The laparoscopic colectomy group had a decreased pennation angle at 2 weeks however this recovered by 4 weeks at 6 weeks was no different to preoperative levels. Although a difference existed between the groups at 2 and 4 weeks the only statistically significant difference was noted at 6 weeks post surgery where pennation angle had decreased by 7% in the open group ($p<0.05$).

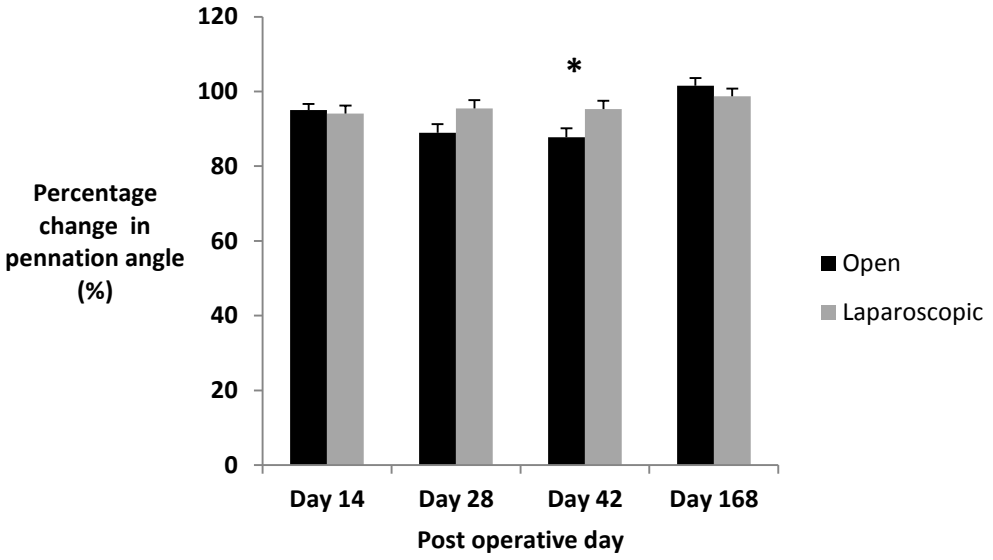


Figure 5.7 Percentage change in pennation angle

*Preoperative values are used as baseline readings (100%). * refers to a statistically significant difference between groups (p<0.05).*

Measurements for fascicle length noted no statistically significant change during the entire study for either group. Neither was there a statistically significant difference noted between the groups.

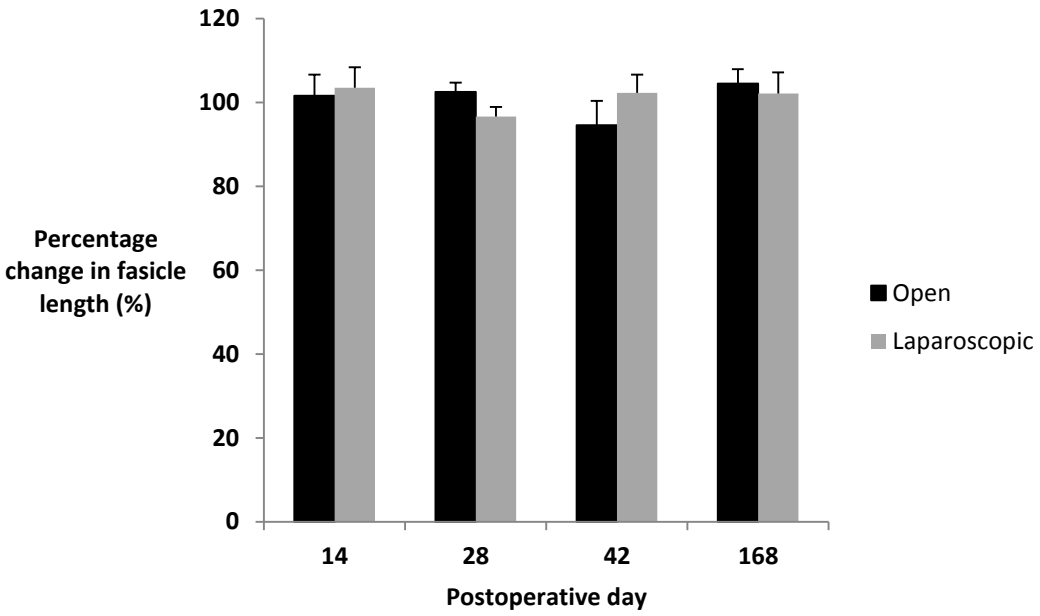


Figure 5.8 Percentage change in fascicle length

Preoperative values are used a baseline readings (100%).

5.4.1.2 Correlations

Correlations between changes in muscle thickness, pennation angle and fascicle length were explored and data presented in figures 5.9, 5.10 and 5.11. A significant positive Pearson product-moment correlation was noted between pennation angle and muscle thickness ($r=0.60$, $p<0.05$) (figure 5.9), however no correlation was noted between fascicle length and pennation angle (figure 5.10) and between fascicle length and muscle thickness (figure 5.11).

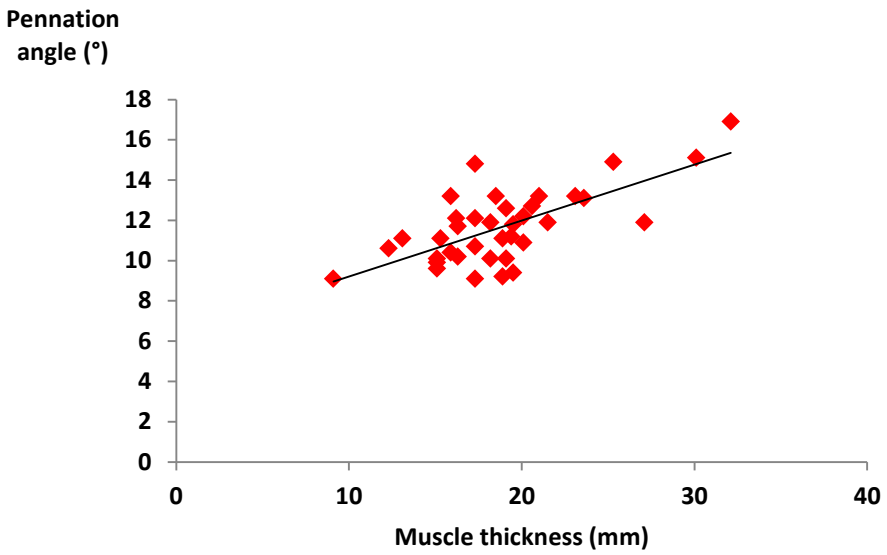


Figure 5.9 Correlation between pennation angle and muscle thickness of *vastus lateralis* at 6 weeks post surgery

The red dots mark results for individual patients. The black line represents the line of best fir (r=0.69, p<0.05).

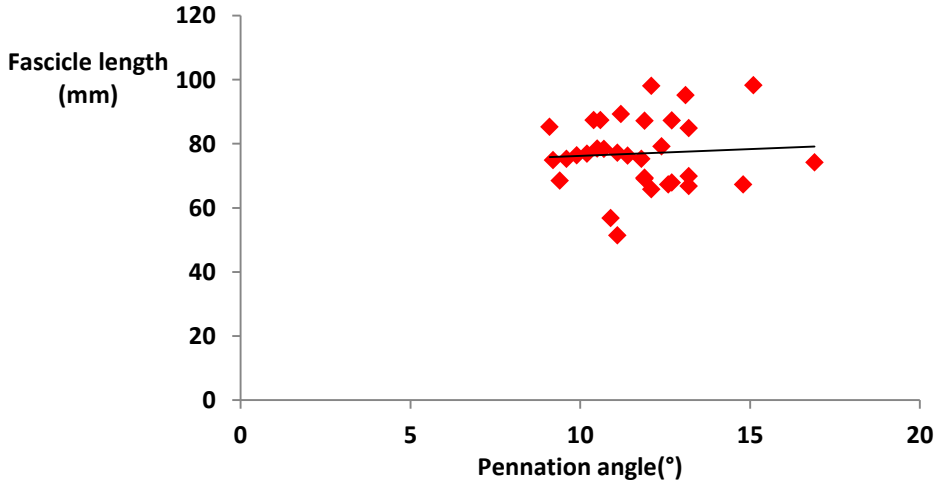


Figure 5.10 Correlation between fascicle length and pennation angle of *vastus lateralis* at 6 weeks post surgery

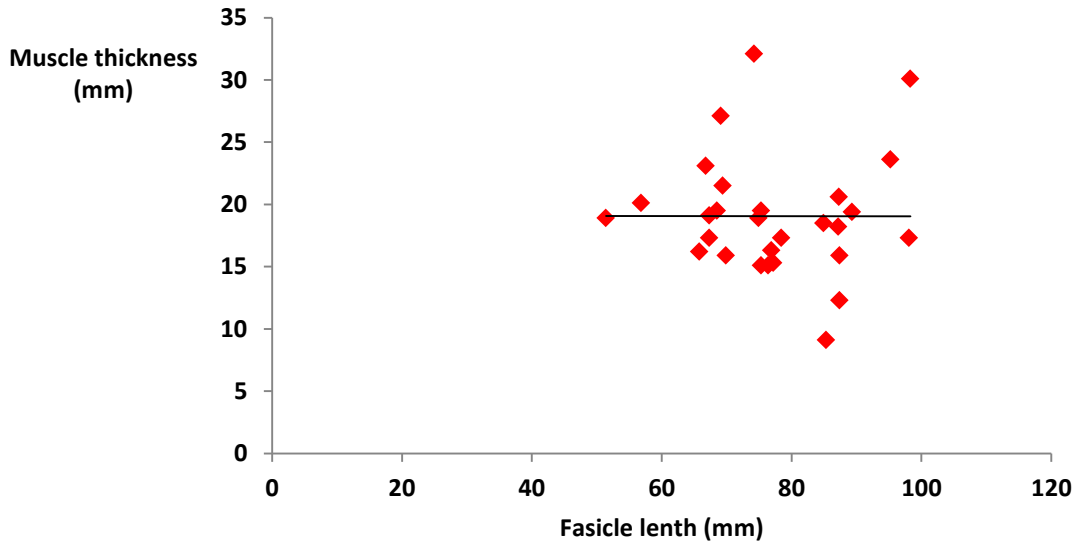


Figure 5.11 Correlation between fascicle length and pennation angle of *vastus lateralis* at 6 weeks post surgery

5.4.2 Muscle protein synthesis rate

Of the 8 participants (4 open and 4 laparoscopic) recruited for this study, results for 6 were analysed. One laparoscopic participant dropped out of the study after their first muscle biopsy due to pain from a small haematoma and one laparoscopic patient was removed from the study after requiring a postoperative blood transfusion (3 units packed red cells). One of the remaining laparoscopic patients was converted to open procedure during surgery due to inability to identify and safely ligate the mesenteric vessels. This left the sub-set population consisting of 5 open and 1 laparoscopic colectomy. Data for the APE *alanine* recovered from muscle biopsies prior to surgery and after 42 days of recovery are shown in table 5.2. The mean adjusted average APE *alanine* was used to calculate the FSR. We did not perform statistical analysis between open and laparoscopic patients.

Muscle biopsy	Participant						Mean
	A	B	C	D	E	F	
Adjustment	-131.5 ±0.3	-131.2 ±0.8	-131.1 ±0.8	-129.5 ±0.7	-137.3 ±0.3	-132.1 ±0.7	
Day 0	-37.5 ±0.7	2.75 ±1.8	LS	28.1 ±0.9	132.4 ±2.5	LS	41.4 ±1.9
Day 42	1847.5 ±2.1	1760.6 ±0.9	1675.1 ±6.8	2149.3 ±20.2	1515.5 ±2.1	1359 ±6.6	1717.8 ±7.2

Table 5.2 Muscle alanine enrichment

Data consists of mean and standard deviation. LS- Persistently low signal.

Plasma D_2O enrichment data of each participant during the study (APE precursor) were used to create mean APE precursor values for each study day. The data is depicted by figure 5.12. The mean overall APE was produced by calculating the area beneath our curve using the trapezoidal method. The average muscle protein synthesis rate was calculated using the linear equation in the method section and equalled 1.05 ±0.02 %/day.

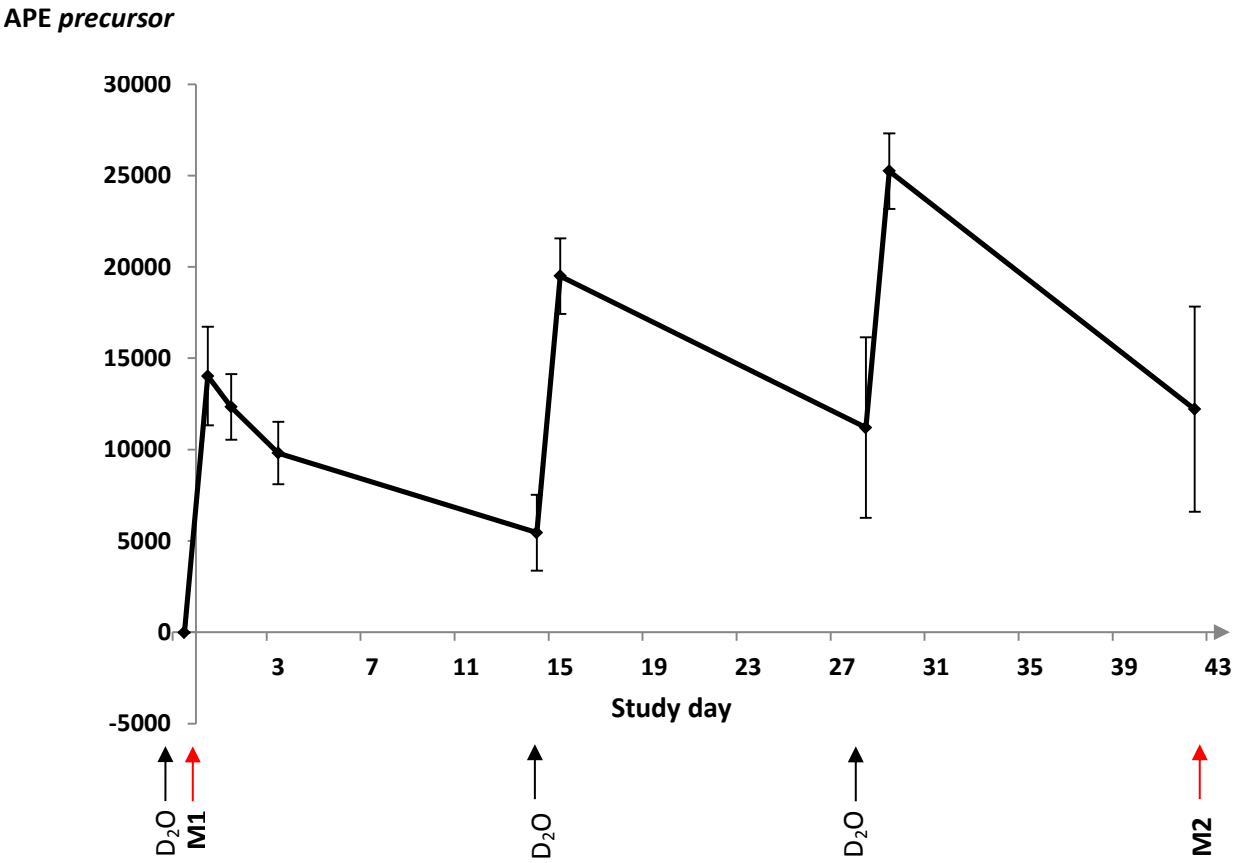


Figure 5.12 Mean plasma enrichment at various time points during study

Line chart depicting mean plasma enrichment and standard deviations during the study. Black arrows indicate deuterium oxide administration (day -1, day 14 7 day 28), red arrows indicate timings of muscle biopsies (M1 day 0; M2 day 42). Day 0 is the day of surgery.

5.5 DISCUSSION

This study showed that B-mode ultrasound characterises changes within skeletal muscle architecture after surgery for colorectal adenocarcinoma and that D_2O use as a bio-label to assess muscle protein synthesis rates over a period of 42 days is feasible.

Assessment of *vastus lateralis* architecture using B-mode ultrasound is a reliable estimate of *vastus lateralis* cross sectional area and hence mass (de Boer, Seynnes et al. 2008). Baseline measurements (prior to surgery) for muscle thickness, fascicle length and pennation angle showed no statistical difference between groups. Therefore we can assume both groups were evenly matched regarding *vastus lateralis* mass, eliminating a potential source of selection bias.

Decreases in muscle thickness during recovery signify either a reduced rate of muscle protein synthesis or increase in the rate of muscle protein breakdown after surgery. Possible explanations include; a catabolic effect produced by circulating inflammatory markers associated with the surgical stress response and/or atrophy caused by prolonged bed rest and reduced activity in the early postoperative period. Variation between the groups is noticeable after 2 weeks and peaked at 6 weeks postoperatively. Although at this point the surgical stress response is dissipating, bed rest and inactivity have been ongoing for more than one month.

Laparoscopic patients suffered only minimal changes in muscle architecture and appear to be protected from excessive muscle mass losses. Laparoscopic surgery has widely been reported to be associated with a shorter, dampened surgical

stress response (Veenhof, Sietses et al. 2011). When this information is combined with the fact that absence of a laparotomy wound is associated with improved ambulation and earlier resumption of daily activities, these results are unsurprising (Bhalla, Williams et al. 2014). After open colorectal surgery there may be an initial increase in muscle protein breakdown associated with the surgical stress response accompanied by inactivity due to postoperative convalescence which produces a degree of muscle atrophy.

The most common and apparent adaptation of skeletal muscle to disuse is a loss of muscle mass reflected in a decrease in size (LeBlanc, Rowe et al. 1995). Healthy volunteer studies in which subjects were immobilised for a period of time also report decreases in muscle size associated with alterations in muscle thickness, fascicle length and pennation angle (Narici and Cerretelli 1998; Kawakami, Muraoka et al. 2000; Reeves, Maganaris et al. 2002). In these studies the period of immobilisation/disuse varied from 20-90 days and muscle mass decreased by between 7-30%.

Several clinical publications report loss of lean muscle mass after surgery, however only one has used B-mode ultrasound. A study by *Venrooij et al* used DXA to quantify the changes that occur after cardiac surgery and noted a 5% decrease in lean muscle mass at 2 months postoperatively (van Venrooij, Verberne et al. 2012). *Jensen et al* also used DXA to quantify postoperative changes in patients undergoing open colorectal surgery, with the authors reporting a loss of 10% lean muscle mass on postoperative day 10 which was still apparent at postoperative day 30 (Jensen, Houborg et al. 2011). Only one study used B-mode ultrasound postoperatively, noting a 30% reduction in lean muscle mass in patients undergoing bariatric surgery which became apparent 4

weeks after surgery and continued for 3 months (Pereira, Marchini et al. 2012). Our study offers the first insight into muscle architectural adaptations after colorectal surgery.

Healthy volunteer studies indicate that the onset of muscle atrophy appears to occur within a few days of disuse and after 7 days of strict bed rest a 3% decrease in thigh muscle volume is observable (Ferrando, Stuart et al. 1995). We found reduced muscle thickness which was first apparent 2 weeks post surgery and continued until week 6. The maximum mean loss of muscle thickness was 8%. Considering muscle thickness correlates well with cross-sectional area and that the length of the muscle (from origin to insertion) is constant the 8% loss in muscle thickness correlates with a similar percentage loss in muscle mass.

Ultrasound-based muscle thickness measurements involve not only muscle but also non-contractile tissues such as intermuscular adipose and connective tissues which increase with aging (Buford, Lott et al. 2012). Whether intermuscular adipose and connective tissues decay at the same rate as skeletal muscle post surgery is unknown, therefore there is the distinct possibility that, for older individuals, B-mode ultrasound measurements for muscle thickness may overestimate lean tissue mass and that the losses within our population may in fact be greater.

Muscle thickness relates to the number and/or size of fibres found within any given muscle. Although we did not measure lower limb strength, studies have noted B-mode ultrasound evaluation of muscle thickness (and hence cross-

sectional area) correlates well with force of contraction (and strength) in healthy volunteers undergoing resistance training (increasing muscle thickness) and bed-rest (inducing atrophy) (de Boer, Seynnes et al. 2008). The loss of strength however is not proportional to the loss of muscle bulk, with several studies noting a loss of 6% cross sectional area producing a decrease in force of contraction of over 25% (de Boer, Seynnes et al. 2008).

Pennation is a strategy to pack greater numbers of contractile elements along the aponeurosis and tendon (Narici 1999). With muscle atrophy the reduction in fibre pennation angle is thought to be indicative of a loss of sarcomeres in parallel (Gans and Bock 1965). The loss of these function contractile units is associated with a reduction of cross-sectional area and general lower limb weakness (de Boer, Seynnes et al. 2008). In our study, although pennation angle decreased after surgery, only at the 6 week mark was there a statistically significant difference between the groups. This study was not powered for variations in muscle architecture and there may be a more pronounced difference between the groups earlier if the sample size was greater.

We did not find a statistical change in fascicle length during this study. Again, this study was not powered for variations in muscle architecture and there may be significant differences between the groups if the sample size was greater. The majority of publications relating to muscle atrophy use a period of strict immobilisation or bed rest, where the participants do not weight-bear at all. Within our study our participants were all patients recovering after colectomies following enhanced recovery programmes which include active physiotherapy which encourages patients to mobilise independently if possible.

A possible explanation for the absence of changes in fascicle length seen in our study could be due to the POM used. The majority of studies using B-mode ultrasound assess muscle architecture by evaluating the response of muscles to exercise/resistance training. In these studies volunteers are usually healthy and young with the POM used varying between 40 to 60% of the vertical distance between the greater trochanter and femoral condyle, thereby focussing the ultrasound probe directly onto the muscle belly.

Studies aimed at measuring muscle atrophy are few, and aim to quantify muscle losses by taking several measurements are various positions down the *vastus lateralis* muscle to accurately estimate the volume of the entire muscle before and after a period of bed-rest/immobilisation. Within our study the subjects are patients with cancer and an average age of 68 years. We focus our attention on taking single snapshots across the *vastus lateralis* muscle at regular intervals over several weeks. Several participants were enrolled in other studies where muscle biopsies were taken from the *vastus lateralis* mid-belly (50% of the distance from greater trochanter to femoral condyle). In order to not influence the ultrasound snapshot we decided to standardise our recordings to a POM 60% of the distance from greater trochanter to the femoral condyle therefore readings could be taken without the presence of scar tissue potentially altering results.

For young healthy volunteers use of a POM at 60% (distance from the greater trochanter to the femoral condyle) does not seem to affect recordings of pennation angle and fascicle length however it may cause a problem in older patients. As individuals age muscle mass naturally decreases (sarcopenia) whilst intermuscular adipose tissue increases making variations in muscle architecture

less accurate the further from the mid-belly you move. In some cases an image taken at POM 60% may include a snapshot across part of the musculo-tendinous junction and not the muscle itself (figure 5.13).

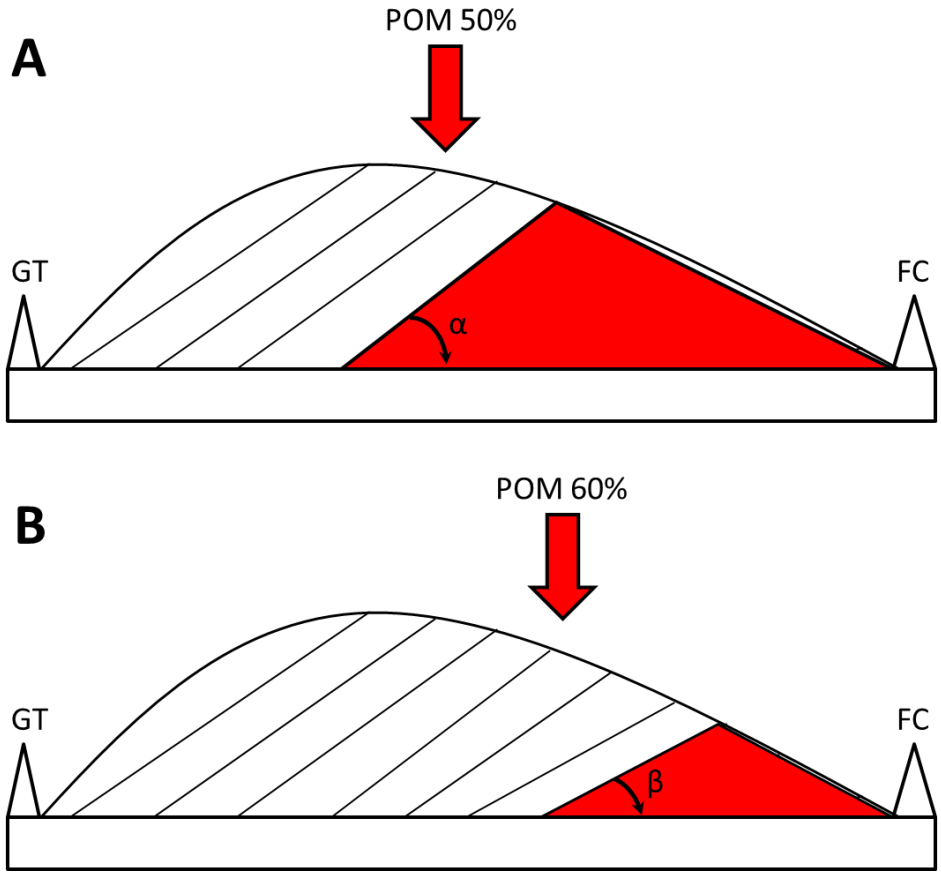


Figure 5.13 Comparison of pennation angle measurements at various different points of measurement along vastus lateralis.

A – Pennation angle (α) noted at point of measurement 50% of distance from greater trocanter (GT) and femoral condyle (FC). B- Pennation angle (β) noted at point of measurement 60% of distance from greater trocanter (GT) and femoral condyle (FC).

Measurements for muscle thickness and pennation angle (changes from baseline) may not be affected if the same POM is used each time however those for fascicle length might be. Within this region the arrangement of fibres is not uniform, unlike the muscle mid-belly. As we move away from the muscle mid-belly the pennation angle decreases. Fascicles in this region appear to arc towards the muscle-tendon junction. If this occurs greater extrapolation is required to estimate length, increasing the potential of producing erroneous results as fascicles and the tendon unify (figure 5.14).

Correlation between changes in vastus lateralis muscle thickness and pennation angle exists (Strasser, Draskovits et al. 2013). As muscle mass increases more fibres are placed on top of each other (in parallel); therefore as muscle thickness increases so does the pennation angle and as muscle is lost both muscle thickness and pennation angle decrease. Within our study we also note a significant positive Pearson product-moment correlation coefficient between pennation angle and muscle thickness ($r=0.69$, $p<0.05$). Studies have described a relationship between pennation angle and fascicle length also (de Boer, Seynnes et al. 2008). As muscle mass is lost and pennation angle decreases fascicle length may shorten. We found no such correlation within our study. We also found no correlation between fascicle length and muscle thickness. Although this study was not powered to detect changes in muscle architecture a possible explanation of these results is that as muscle is lost from *vastus lateralis* and the pennation angle becomes more acute, muscle fibres may lengthen in order to mitigate the loss of sacromeres in parallel by re-placing them in series.

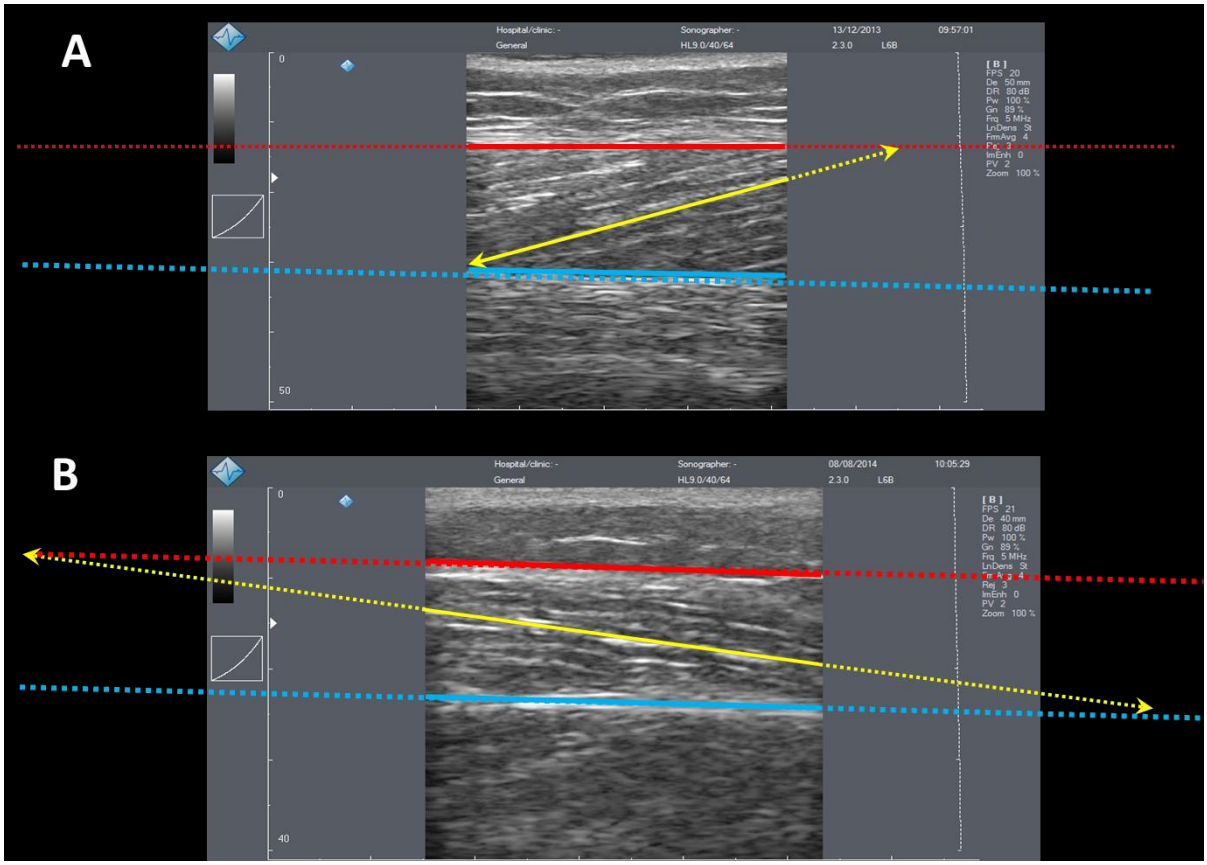


Figure 5.14 Extrapolation comparisons between various points of measurement

A-The POM is close to the muscle mid-belly as the pennation angle is large and only minimal extrapolation is required to measure fascicle length. B- The POM is further from the muscle mid-belly with a more acute pennation angle and greater extrapolation required to measure fascicle length. Red line- superficial aponeurosis; Blue line- deep aponeurosis; Yellow line- muscle fascicle. Dotted lines represent extrapolation using Image J.

We successfully maintained deuterium oxide body water enrichment in patients undergoing colorectal cancer surgery for 42 days. This is the first study to maintain enrichment for this length of time within a patient population and the first to include the period of surgery. Every subject was successfully loaded with deuterium oxide and each had data collected charting the enrichment of body

water for the entire length of the study. Increased levels of alanine containing deuterium was found within every muscle biopsy at day 42.

Use of D_2O to measure turnover in skeletal muscle has been well validated in healthy volunteers (Gasier, Riechman et al. 2011; Robinson, Turner et al. 2011; Gasier, Fluckey et al. 2012; MacDonald, Small et al. 2013; Wilkinson, Franchi et al. 2014). The majority studies compare skeletal muscle turnover in response to anabolic stimuli with the longest period of assessment being 4 weeks (exercise). In-house data from our collaborators recorded MPS rates of 1.46±0.06%/day in the non-exercising leg of healthy volunteers over an 8 day period (Wilkinson, Franchi et al. 2014). We recorded a mean MPS rate of 1.05±0.02 %/day from our patients over 42 days. If we use this data to review the drop in MPS rate seen post surgery we note a 28% drop.

We must be cautious however, as these healthy volunteers are not age matched controls. Muscle mass decreases with age (sarcopenia) due to blunting of MPS rates which usually rise after feeding which allows MPB to occur relatively unopposed. A second consideration is the fact that by definition these healthy volunteers do not have cancer. Cancer requires energy to progress with the majority of solid organ tumours associated with changes in body composition leaning towards cachexia. A recent study on patients suffering from gastrointestinal cancer (oesophagogastric) has reviewed MPS rates (using deuterium bio-labelling over 7 days) in age matched controls, patients with cancer who are weight neutral and those with cancer who are cachectic (MacDonald, Johns et al. 2015). The authors report MPS rates of 1.39%/day in healthy volunteers, 1.46%/day in weight neutral patients suffering from gastrointestinal cancer and 1.75%/day in patients suffering gastrointestinal

cancer who were cachectic. None of these groups had an operation during the study. Comparing our results with these controls we note a 24%, 28% and 40% drop in MPS rates seen over the 42 days post surgery.

Unfortunately we were unable to compare open and laparoscopic MPS rates due to procedure specific complications. Previously publications report that laparoscopic surgery may help to resist the drop in MPS normally associated with open surgery (Essen, McNurlan et al. 1993; Petersson, Hultman et al. 1995; Caso, Vosswinkel et al. 2008). The majority of these studies use traditional amino-acid labelling techniques. The major problem with this technique is that by administering labelled amino acids there is always the risk of stimulating MPS directly.

Essen et al combined deuterium bio-labelling with 24 hour amino acid flooding techniques to quantify the changes occurring within the first 24 hours post surgery (Essen, Thorell et al. 1995). The authors noted a 28% drop in MPS rate (1.77±0.11 v 1.26±0.8%/day) for open cholecystectomy compared to a 20% drop in laparoscopic cholecystectomy (1.97±0.15 v 1.57±0.15%/day) after 24 hours. This provides evidence to support a drop in MPS rate with open procedures. Cholecystecomy is a shorter, less traumatic operation compared with colonic resection. We also found a 28% drop in MPS rate however our results encompass 42 days post surgery. It could well be the case that the drop in MPS rate 24 hours post colectomy is far greater.

Previous publications have quantified that a reduction in MPS rate may last for up to 30 days post abdominal surgery (Petersson, Wernerman et al. 1990).

Petersson et al noted that elective abdominal surgery caused a sustained depression of protein synthesis for over 30 days using percutaneous muscle biopsies taken at various time points and determining protein synthesis rates by assessing the total concentration and size distribution of ribosomes. They report the mean total concentration of ribosomes per milligram of DNA decreased by 27.5% ($p<0.07$), 44.5% ($p<0.007$), 48.3% ($p<0.007$) and 45.0% ($p<0.07$) on days 3, 10, 20 and 30, respectively. Our findings indicate that the period of dampened MPS may last more than 42 days despite use of enhanced recovery programs which were not in use at the time of these studies.

Deuterium was tolerated in every patient. Previous in-house studies involving D_2O boluses highlighted administration in older patients may produce a degree of nausea and/or dizziness. This is presumed to be due to a vestibular-ocular disturbance. We noted two volunteers felt nauseous after their first dose. Onset occurred 30 minutes after ingestion and lasted for 1 hour. Both patients tolerated second doses better by splitting the doses in half and administering them with separately within a 10-20 minute window. We did have one problem with a muscle biopsy site. The biopsy was taken prior to surgery whilst the patient was under anaesthesia. Unfortunately the patient complained of pain 2 weeks post procedure due to a haematoma. This is unusual but probably due to administration of high-dose clexane (40 milligrams, sub-cutaneous) as prophylaxis against the development of deep vein thrombosis. This patient refused a second muscle biopsy and left the study.

A particular strength of this study was the visualisation and quantification of muscle architectural changes with B-mode ultrasound. The reason for using B-mode ultrasound rather than DXA, CT or MRI was to improve overall compliance

by bringing equipment to the patient rather than asking the patients to visit various different parts of the hospital. B-mode ultrasound allowed us to monitor changes every 2 weeks, enabling us to pinpoint differences between open and laparoscopic recovery pathways; something no previous study has been able to achieve.

5.6 CONCLUSION

We have shown B-mode ultrasound can be used to monitor changes in muscle architecture within the postoperative period. There is a greater decrease in thickness and pennation angle of *vastus lateralis* after open colonic cancer surgery than for laparoscopic surgery. We note a 28% reduction in MPS rates during the first 6 weeks post surgery. Stimulation of MPS prior to surgery may offset postoperative reductions in muscle mass.

Chapter 6

Functional recovery

6.1 INTRODUCTION

Colorectal cancer surgery is associated with a period of recovery which begins in hospital and continues after discharge. Standard definitions of what constitutes 'recovery' in the postoperative context are inconsistent, and the word has been applied to physiological, psychological, social and habitual dimensions in quantitative and qualitative research studies (Allvin, Berg et al. 2007). The only consensus regarding the definition that can reasonably be agreed upon is that it is not synonymous with discharge from hospital (Smart and Daniels 2013). Despite this, length of hospital stay has remained as a standard outcome measure in surgical research because of its ease of measurement and impact upon health economics and resource utilisation in secondary care.

An alternative outcome measure is time taken to resume 'activities of daily living'. Although vague, difficult to measure and open to interpretation, this may in fact give us a better estimate of the impact of surgery upon an individual, where resumption of daily duties signifies complete recovery. Knowledge of the post discharge period is limited and often based upon information gained from studies focused upon quality of life where self-assessment tools give an insight into physical and psychological functioning without proving data referring to changes in physical performance.

A method commonly used to assess physical performance is muscle strength. Hand grip is the simplest method available to measure muscle strength and function in clinical practice (Roberts, Denison et al. 2011). In elderly postoperative patients, hand grip strength has been shown to accurately predict

survival and long term functional decline (Bohannon 2008; Chen, Ho et al. 2011; Garcia-Pena, Garcia-Fabela et al. 2013).

Given that the majority of recovery in the era of enhanced recovery programs takes place outside of the hospital, there is a need for greater knowledge regarding functional recovery with specific tools able to assess physical performance, quality of life and the ability to perform daily activities.

6.2 AIMS

1. To map out the postoperative period after colon cancer surgery with particular focus upon functional recovery.

2. To compare the recovery profile of open and laparoscopic colectomy patients

3. To compare objective markers of physical function with subjective patient reported assessments of functionality.

6.3 METHODS

6.3.1 Hand grip strength

Limb strength is far easier to measure than axial strength in participants of any age. For this study we use hand grip strength for several specific reasons. Firstly hand grip strength has been used in numerous population and patient based studies with significant predictive validity (Schwenk W 2005). Low values are associated with increased risk of falls, disability, impaired health-related quality of life, prolonged length of stay in hospital and increased mortality in elderly

patients (Sayer, Syddall et al. 2006; Gale, Martyn et al. 2007; Syddall, Martin et al. 2009). In elderly postoperative patients, hand grip strength also accurately predicts specific outcomes including survival, institutionalization and long-term (>6 months) functional decline (Bohannon 2008; Chen, Ho et al. 2011; Garcia-Pena, Garcia-Fabela et al. 2013).

Secondly, a hand grip dynamometer is portable allowing easy assessment in both in-patient and outpatient settings. This allows regular assessment with little impact upon clinical care thereby improving participant compliance. Lastly in patients post abdominal surgery measuring leg strength in the immediate postoperative period is very difficult. Extension of the knee requires contraction of the quadriceps muscles which originate from the pelvis. Incisions used in both open and laparoscopic colectomy may extend toward the pubic symphysis and thus extension of the knee may be limited by pain from an incision situated in close proximity to the quadriceps origin.

Hand grip strength was assessed using a grip strength dynamometer (Taeki Scientific Instruments, Niigata-City, Japan) on each study day. Patients were instructed to keep their shoulders adducted and neutrally rotated with the arm in a vertical position, the wrist in a neutral position and then to squeeze the grip with maximal strength using their dominant hand (figure 6.1). This was repeated three times in a seated position and the highest result obtained recorded.

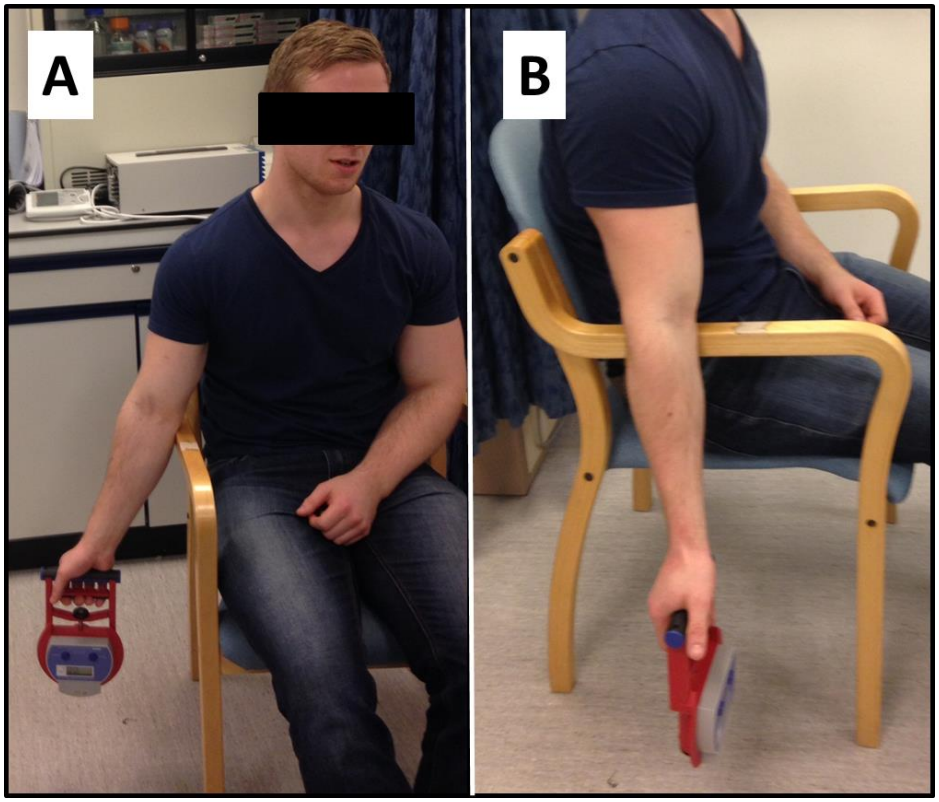


Figure 6.1 Participant using hand grip dynamometer

A- forward-facing view; B- lateral view

6.3.2 Pain

Pain was assessed using a nominal pain rating scale (see appendix III). The scale consisted of a 10cm line with the numbers 0-10 marked exactly 1cm apart. Patients recorded their perceived pain score at that particular time on each study day. A score of '0' represents no pain whilst a score of '10' represents the worst pain imaginable.

6.3.3 Dukes activity status index

Thorough investigation of postoperative recovery requires a measure of functionality. We chose to use a subjective assessment of functionality with the

DASI questionnaire (see appendix III). This questionnaire is composed of specific questions focussed upon an individual's lifestyle and quantifies functionality by giving researchers a subjective score and a validated estimate of $V0_2$ max (Hlatky, Boineau et al. 1989).

The Duke Activity Status Index (DASI) is a 12-item questionnaire where the answers can only be 'yes' or 'no'. Each question carries a specific score for a 'yes' reply and a score of '0' for a 'no'. The questions focus on functional capabilities based upon activities of daily living and some recreational activities. Questions assessing more strenuous activities carry greater scores. Questions left blank are scored as '0'. Once completed the scores for 'yes' answers are added together to create the DASI score. This DASI score is incorporated into the following equation to produce a validated estimate of maximal oxygen consumption (Hlatky, Boineau et al. 1989).

$$VO2\ Max\ (ml/kg/min)\ =\ (0.43\ x\ DASI\ Score)\ +\ 9.6$$

6.3.4 EQ5d5l

A measure of health status is important for understanding why changes in functionality occur. We chose EQ5d5l because it enables patients to score their perceived health (VAS) and also gives researchers valuable clues as to why patients have attributed themselves with a certain score (5 dimension scale) (see appendix III).

EQ-5d is a standardised measure of health status developed by the EuroQol Group in order to provide a simple, generic measure of health for clinical and economic appraisal. EQ5d5l, the English version has been well validated for use in patients both in hospital and in the community (Brazier, Jones et al. 1993; Parkin, Rice et al. 2004). It consists is two pages, the EQ5d5l descriptive system (page 1) and the EQ Visual Analogue scale (EQ VAS) (page 2). The descriptive system comprises 5 dimensions (mobility, self-care, usual activities, pain/discomfort, anxiety/depression). Each dimension has 5 levels: no problems, slight problems, moderate problems, severe problems, and extreme problems. The respondent is asked to indicate his/her health state by ticking (or placing a cross) in the box against the most appropriate statement in each of the 5 dimensions.

The EQ VAS records the respondent's self-rated health on a 20 cm vertical, visual analogue scale with endpoints labelled 'the best health you can imagine' and 'the worst health you can imagine'.

6.4 RESULTS

6.4.1 Strength

Preoperative values for hand grip were similar in both groups (open surgery 35.54±10.1 v laparoscopic surgery 33.45±9.5, $p=0.83$). Postoperatively hand grip decreased for the first two days reaching a low at Day 2 (open surgery 27.93±8.3 v laparoscopic surgery 28.94±9.1, $p<0.01$). After this point hand grip strength begins to increase with laparoscopic surgery associated with a return to

baseline at day 42 whilst hand grip strength failed to reach preoperative values for the open group 6 months post surgery (figure 6.2).

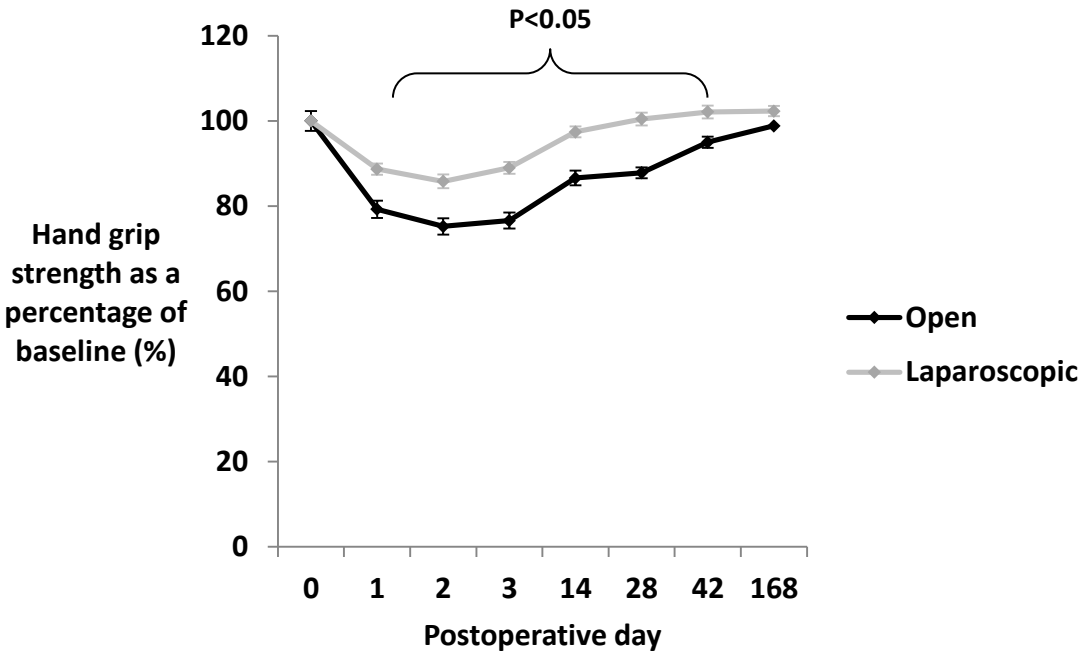


Figure 6.2 Mean changes in hand grip strength

Preoperative values are used a baseline (100%). Data presented as mean±SEM. Statistically significant differences between the groups are marked (independent samples t-test).

6.4.2 Pain

Neither group exhibited significant preoperative pain; however Day 1 post surgery the open group median pain score was 6 compared to the laparoscopic group score of 4. Pain scores decreased in both groups until day 3. At 2 weeks post surgery the median pain score was 0 in the laparoscopic group and 1 in the

open group. At 6 months the score reached baseline levels in both groups (figure 6.3).

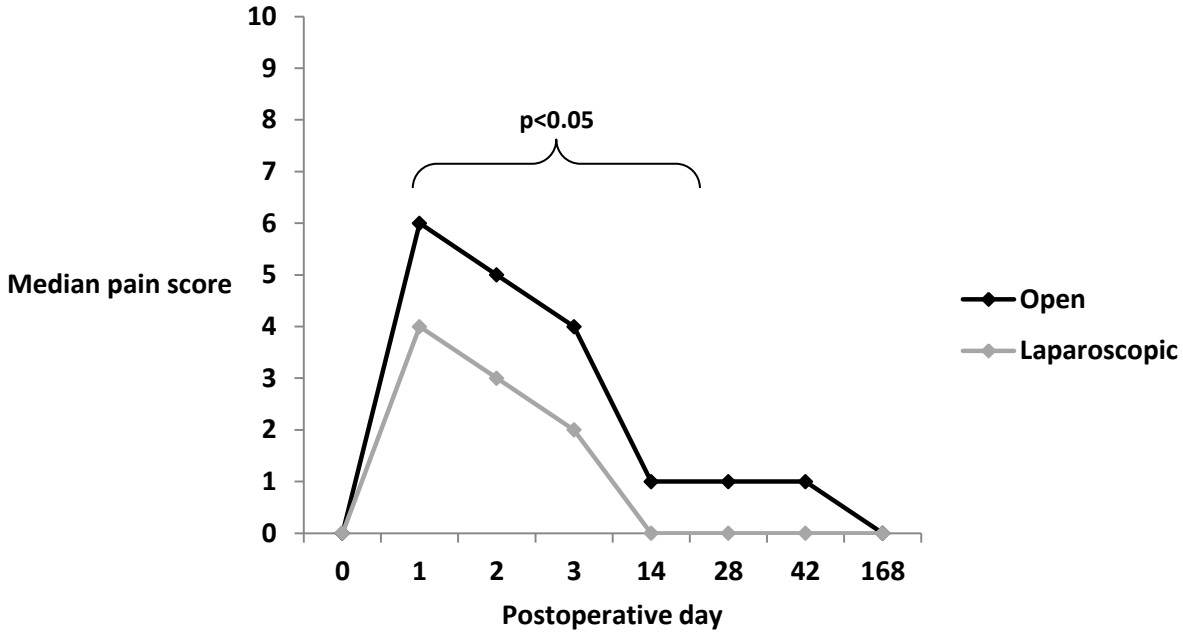


Figure 6.3 Median pain scores

Statistically significant differences between the groups are marked (Mann-Whitney U).

6.4.3 Activity status

Mean DASI score preoperatively was 34.9±8.5 for the open and 33.4±9.1 laparoscopic groups respectively (figure 6.4). Two weeks post surgery mean scores dropped by 51% and for the open group and 49% for the laparoscopic group (p=0.82). Mean DASI scores increased at 4 and 6 weeks post surgery and statistically significant differences where noted between the groups (p<0.05). By 6 months the open group had not returned to preoperative values (OS 93.9% v LS 98.3%, p<0.05).

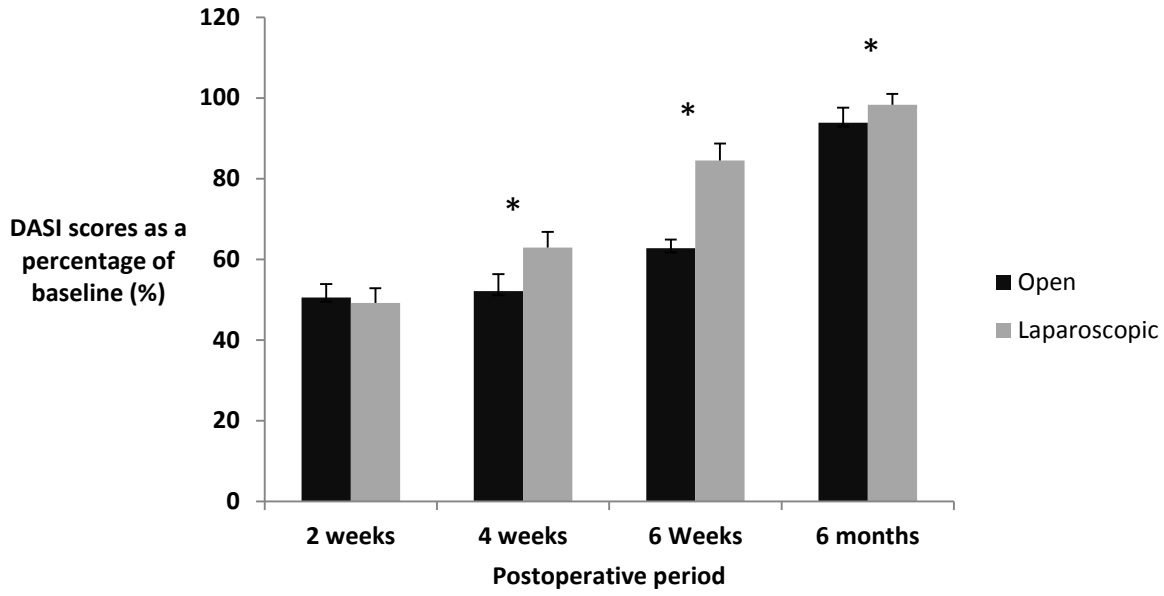


Figure 6.4 Dukes activity status index scores presented as a percentage of baseline values

*Preoperative data were used as baseline values (100%). Data presented as mean±SEM. * Highlights statistically significant differences between the groups (p<0.05, independent samples t-test).*

6.3.4 Health and well-being

Results for the EQ5d-5L are presented by figures 6.5 & 6.6. Mean preoperative VAS score for overall health was 95.9±0.7% and 93.3±0.9% for the open and laparoscopic groups respectively. No statistically significant difference was noted between the groups. At two weeks post surgery the scores had dropped to 75.5±1.7% for the open group and 82.2±1.4% for the laparoscopic group and a statistically significant difference was noted (p<0.05). Scores improved steadily

thereafter, however significant differences existed between the groups at day 28 and 42. No difference was noted at 6 months.

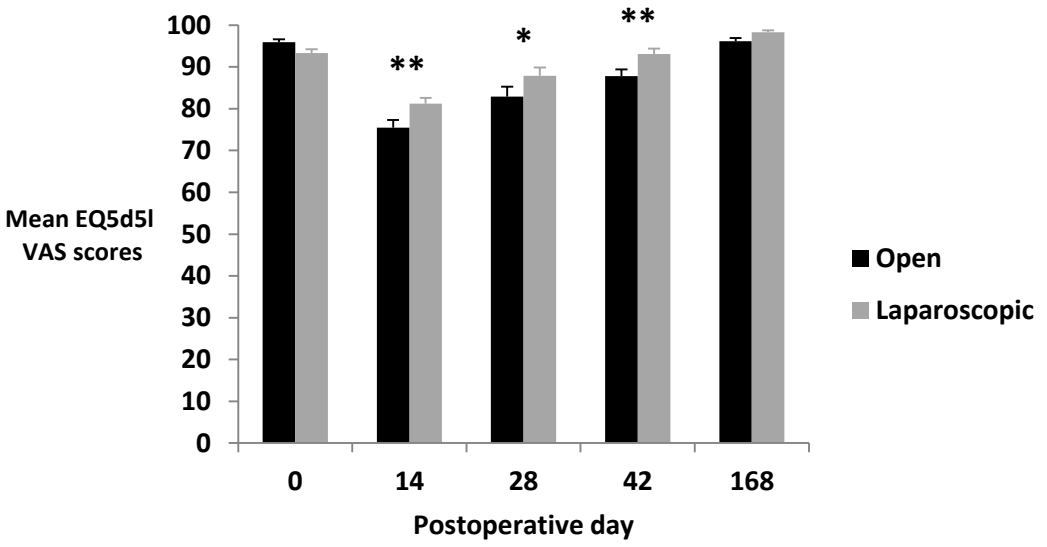


Figure 6.5 Mean EQ5d5l visual analogue scores

*Data presented as mean±SEM. * Highlights statistically significant differences between groups (p<0.05, independent t-test). ** Highlights statistically significant differences between the groups (p<0.01, independent samples t-test).*

Looking at the 5 dimension responses it becomes apparent there is a degree of pre-operative anxiety within both groups of patients (22.2% open surgery v 26.9% laparoscopic surgery) (fig 6.6). Reported problems with mobility, self-care, inability to perform usual activities and pain were equal between the groups. At two, four and six weeks post surgery there was a large increase in reported problems associated with pain and the ability to perform usual activities especially in the open group. By 6 months there were few reported problems in any domain.

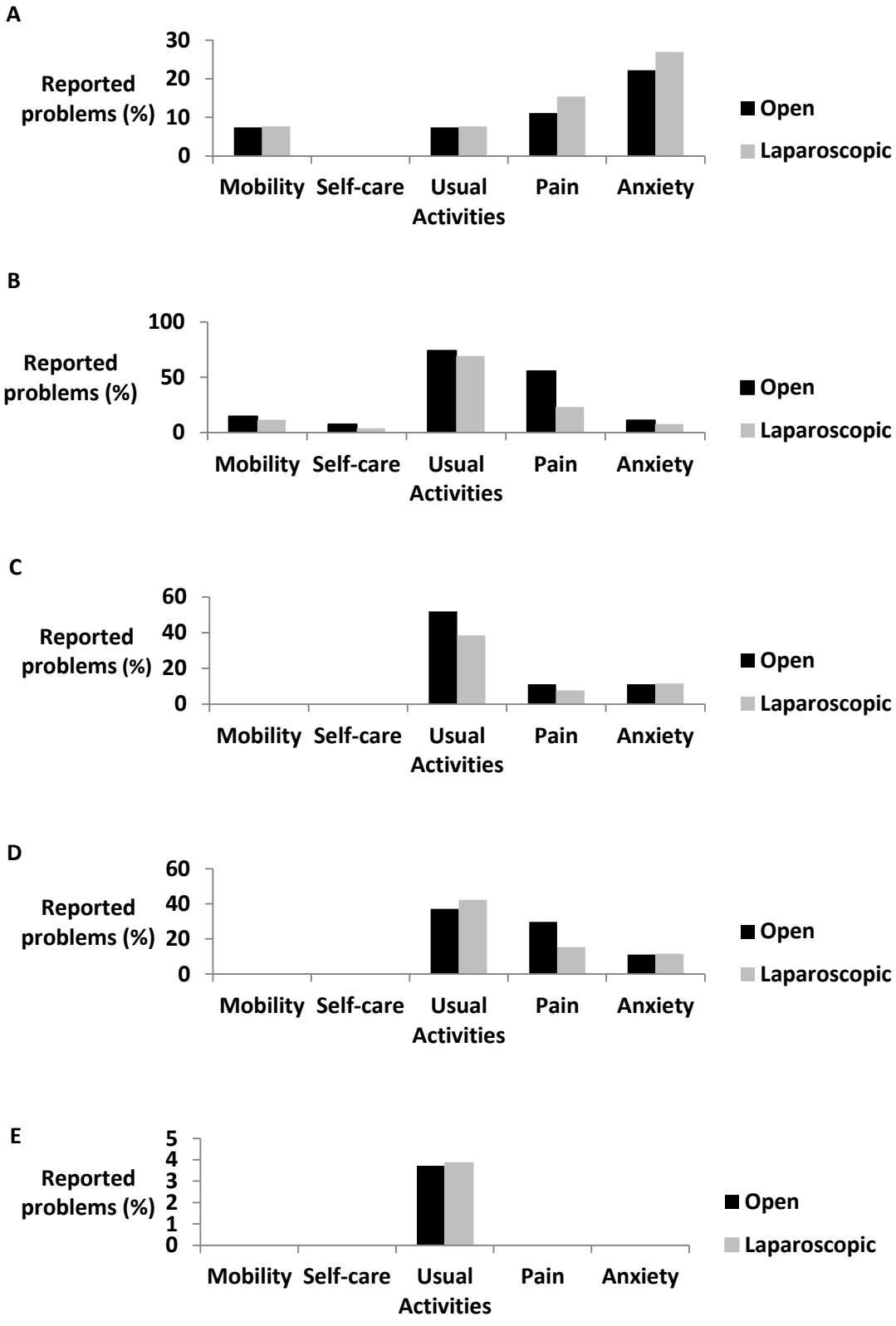


Figure 6.6 Health related quality of life from EQ5d5l

A-preoperative, B- 2 weeks postoperative, C- 4 weeks postoperative, D- 6 weeks postoperative , E- 6 months postoperative

6.4 DISCUSSION

This study reports functional aspects of recovery after open and laparoscopic colonic cancer surgery at regular intervals over a 6 month period. We note significant differences in strength, pain, exercise ability and health related quality of life between patients having undergone laparoscopic and open surgery over the first 6 weeks. Minimally invasive surgery was associated with a return to baseline function between 4 and 6 weeks postoperatively whilst full-recovery from open surgery may take up to 6 months.

Recovery of strength in the postoperative period, recorded with a hand-grip dynamometer differed greatly depending upon surgical technique. Open surgery was associated with a significant drop in hand grip strength compared to laparoscopic surgery. Open surgery was associated with a reduced hand grip of almost 30% at day 2; recovering slowly over the next 6 weeks. At 6 weeks, levels were still below preoperative values. Laparoscopic surgery was associated with significantly less postoperative weakness (approximately a 15% decrease) with hand grip strength returning to preoperative levels by 6 weeks.

Two potential mechanisms may account for the greater drop in hand grip strength noted in the open group. Firstly, a greater degree of postoperative muscle modulation may cause excessive muscle mass losses resulting in increased weakness. Secondly, pain stimulated by inadvertent contraction of the abdominal wall musculature whilst squeezing the dynamometer may also account for the drop in strength recorded from the open group. Regardless of the reasons for the drop in hand-grip strength, these patients are not as they were preoperatively and are unable to grip with the same strength as

laparoscopic patients. This weakness will impact upon their ability to perform daily activities post discharge.

Hand grip strength measurements at 6 months post open surgery note a slight decrease compared to preoperative values. At this time point the laparotomy wound has completely healed and the majority of patients are pain-free. The excessive muscle modulation associated with open surgery may not be completely resolved by 6 months. As stated previously, hand grip strength has been shown to have significant predictive validity with low values associated with increased risk of falls, disability, impaired health-related quality of life, prolonged length of stay in hospital and increased mortality in elderly patients (Sayer, Syddall et al. 2006; Gale, Martyn et al. 2007; Syddall, Martin et al. 2009). In elderly postoperative patients, hand grip strength also accurately predicts specific outcomes including survival, institutionalization and long-term (>6 months) functional decline (Bohannon 2008; Chen, Ho et al. 2011; Garcia-Pena, Garcia-Fabela et al. 2013).

Whilst others have noted reduced hand grip strength in elderly patients at discharge and at one month post open colorectal surgery no study has tracked changes at regular intervals over the immediate, short and long term; and no single study has compared the changes that occur between patients undergoing open and laparoscopic colorectal surgery (Andersson, Ansari et al. 2013).

Patients from both groups were pain-free preoperatively however their experiences of pain were significantly different in the postoperative period. Laparoscopic patients had significantly lower pain scores over the first three

days in hospital. Despite prescribed analgesia, median pain scores registered for the open group was six out of ten. Postoperative analgesic control is a particular problem due to the presence of the laparotomy wound. At 24 hours post operation the effects of transverse abdominal plane blockade or spinal anaesthesia has dissipated. Postoperative morphine sulphate dispensed via a patient-controlled infusion allows patients to control the administration of analgesia, however many patients are reluctant to use it frequently due to significant sedentary side effects.

During the first few days patients are encouraged to mobilise with physiotherapist support. Although necessary, physical exertion is associated with a degree of pain and discomfort, especially amongst the open surgery group. Our questionnaires were completed by patients after morning ward-rounds and after they had washed, changed and participated in physiotherapy. These processes all require patients to mobilise (ie. from bed to chair) and may explain why our open surgery patients experienced more pain than expected despite being in hospital and being prescribed analgesia.

After 2 weeks minimal pain was experienced by both groups, with higher values found within the open group (not significant). This may be surprising at first however, when reviewed with health reported quality of life data we note open surgery patients report poorer mobility and may consciously restrict movements in order to avoid pain. Open patients may also require greater pain relief upon discharge than the laparoscopic surgery group, something not measured within this study but potentially playing an important part in recovery.

Reduced pain scores after laparoscopic colorectal surgery have been reported previously. A Cochrane meta-analysis of 2005 collated evidence from 25 randomised controlled trials and noted that at 4 weeks postoperatively a statistically significant difference existed for perceived pain between laparoscopic and open surgery (Schwenk W 2005). We note that open surgery was still significantly more painful than laparoscopic surgery at the 6 week mark.

Various scales and/or scoring indices allow quantification of an individual's ability to perform the activities of daily living; however the majority have only been validated for use in elderly patients allowing assessors to predict the likelihood for autonomous living arrangements. DASI is unique, having been validated for use in patients of any age and being able to measure an individual's functional capacity and estimate their peak oxygen uptake.

DASI scores dropped dramatically in both groups postoperatively. At 2 weeks reported mean DASI scores were 23 points lower than preoperative values, however no significant differences were noted between groups. This may be due to patients being discharged with strict advice regarding lifting and the undertaking of rigorous domestic duties for 4-6 weeks. If patients adhere to this advice they will be unable to gain any marks on 5 of the 12 questions making up the DASI questionnaire and limit the maximum achievable mark. The rigid nature of the DASI questionnaire somewhat impacts upon its use in the immediate postoperative period and potentially masks a significant difference in VO_2 Max between the groups at this time point. Despite this we feel that for our purposes (to review/assess activity regularly over the postoperative period) this is still the best assessment tool available to us.

Between 2 and 6 weeks there was a statistically significant difference in DASI scores between the groups. Open surgery patients appear to be less willing or less able to complete some of the tasks evaluated in the questionnaire. This is supported by data collected for health reported quality of life where at 4-6 weeks there is a marked difference in mobility between the groups. These significant differences are produced by restrictions associated with the laparotomy wound. Poor mobility is probably a combination of physical and psychological processes. Pain from the wound restricts movement however fear associated with a potential wound problem caused by early overuse (ie. hernia, infection, dehiscence) may also be relevant.

Reduced DASI scores are apparent within the open surgery group at 6 months. By this stage patients are pain-free, all sutures have dissolved and wound remodelling has occurred. This may highlight a general deterioration in fitness initially attributed to the restrictive nature of the laparotomy wound which has failed to recover with time. The initial restrictive effects of the wound could also impact upon a patient's confidence to perform demanding labour or exercise in the future.

The decline in the activities of daily living seen in this study mirror those reported in previous studies. *Rønning et al* reviewed functional status after colorectal cancer surgery but confined recruitment to patients over 60 years of age with no comparison between operative techniques (Ronning, Wyller et al. 2014). Their group noted statistically significant decline in the activities of daily living at one month post surgery. A second study by *Amemiya et al* also found a proportion of patients exhibited a decline in the activities of daily living at 6 months; however this study was confined to patients over 75 years of age and

again differences in operative technique were not accounted for (Amemiya, Oda et al. 2007).

Improved health-related quality of life after laparoscopic surgery is associated with less blood loss, better immune and inflammatory responses, less pain and analgesic requirements, faster postoperative recovery of bowel function, food intake, and physical activity (Schwenk and Kehlet 2004; Braga, Vignali et al. 2005). Reviewing our data for health related quality of life, the visual analogue scale scores highlight significant differences between laparoscopic and open surgery. At 2, 4 and 6 weeks there was a statistical difference between groups, with open surgery associated with a poorer patient reported health status than the laparoscopic group. This indicates that open surgery has a larger impact on a patient's perceived health status than laparoscopic surgery.

Analysis of the health status breakdown prior to surgery shows minimal differences between the groups. Of interest almost 20% of patients report a degree of anxiety/depression at this time point. This result is unsurprising as patients completed this questionnaire after being counselled by the preoperative assessment nurses prior to surgery. Two weeks postoperatively patients reported problems in all areas investigated, however the noticeable difference between the groups was postoperative pain. This correlates with information gained from pain scores. Four and 6 weeks post surgery poor health related quality of life appears to be attributed to the inability to conduct the usual activities of daily living, with open surgery associated with a greater proportion of patients highlighting problems compared with laparoscopic surgery. This information mirrors the DASI scores found at these time frames.

Several studies have investigated outcomes including quality of life after colorectal cancer surgery to review the impact of minimally invasive techniques. The majority of studies are based on longer follow up periods (>3 years) and suggest no difference in outcomes (Murray, Lourenco et al. 2006; Dowson, Cowie et al. 2008; Bartels, Vlug et al. 2010). Few studies have researched health related quality of life in the early postoperative period, when differences between surgical approaches are potentially greatest. There have been four studies to date, the first of which reported no difference between groups at 2 and 6 weeks (King, Blazeby et al. 2006).

The second study, by *Janson et al*, used colorectal cancer specific health related quality of life questionnaires (EORTC QLQ-C30) (Janson, Lindholm et al. 2007). They demonstrated statistically significant benefits of laparoscopic surgery at 2 and 4 weeks postoperatively but noted no difference between the groups at 12 weeks. The third study, by *Weeks et al*, used the generic SF36 questionnaire and found statistical differences at 2 weeks only, whilst the fourth by *Jordan et al*, reviewed health related quality of life after colorectal cancer surgery using both a generic (EQ5d5l) and colorectal cancer-specific questionnaire (EORTC QLQ-C30) (Weeks, Nelson et al. 2002; Jordan, Dowson et al. 2014). The findings of this study echo our own; by providing support for improved health-related quality of life outcomes with laparoscopic surgery in the first 4 weeks postoperatively.

A particular strength of our study is the use of both functional status and physical performance measures as outcome variables. We were able to repeat measures at regular intervals encompassing the immediate postoperative phase of recovery in hospital together with the short and longer term recovery periods

after discharge. Our data represent an important addition to current knowledge about the postoperative functional trajectory in this patient group.

The choice of questionnaires used is important. Each questionnaire was selected to obtain specific information with minimal overlap of information. Every questionnaire used was well validated and extremely user friendly. We specifically chose EQ5d5l ahead of other questionnaires for quality of life assessment because it was considerably shorter than the equally well validated EQRCT and SF36. This brevity enabled participants to concentrate and accurately complete this well worded short questionnaire. This is especially important if the same questionnaire is used on several study days. The specific use of a DASI questionnaire compared to an objective measure of functionality (ie. 6 minute walk test, step-test or cardio-pulmonary exercise testing testing) potentially improved compliance. Previously, we attempted to complete a physical test and found it very difficult within the early postoperative period. In fact the problems in attempting to complete these tests upset patients and led to participants dropping out of other studies. It is difficult for patients to repeat physical exercise regularly in the immediate and short term period post abdominal surgery.

A potential limitation of our study was the exclusion of specific cancer related questionnaires to review health status. We felt that to reduce the risk of drop-out post surgery we needed to keep the questionnaires short and the number of questions used down to a minimum. The DASI and EQ5D questionnaires asked different questions of the patients and including a separate cancer specific questionnaire (the majority of which are much longer and contain similar questions) would take up too much time and the repetitive nature of some of the

questions may increase the drop-out rates and reduce the quality of answers given.

6.6 CONCLUSIONS

Laparoscopic colonic surgery is associated with less reduction in strength, less pain, more activity and a better health related quality of life than open colonic surgery in the first 6 weeks of recovery. A proportion of patients undergoing open surgery fail to return to baseline function after 6 months. More must be done to reduce these functional declines, especially for patients undergoing open colorectal surgery.

Chapter 7

Return to work 1 year after surgery for colorectal cancer

7.1 INTRODUCTION

The most demanding aspect of daily living is employment. Throughout Europe, life expectancy has increased, leading to higher age at pension and longer time in the workforce (Carlsen, Harling et al. 2013). In the United Kingdom one quarter of patients diagnosed with colorectal cancer are under the age of 65, with the age for state pension eligibility due to rise to 67 this will significantly increase the number of patients of working age diagnosed with colorectal cancer (NICE 2011). Employment is the key determinant of socio-economic status. For patients diagnosed with colorectal cancer whilst in employment, full recovery including return to work is vital in maintaining socio-economic status. Several studies have reported a relationship between low socio-economic status and reduced colorectal cancer survival rates (Frederiksen, Osler et al. 2009; Cavalli-Bjorkman, Lambe et al. 2011).

Research relating to return to full-time employment for patients with colorectal cancer is limited, however published return to work rates range from 60-89% depending upon time since diagnosis, definition of return to work and severity of disease (Sanchez, Richardson et al. 2004; van den Brink, van den Hout et al. 2005; Gordon, Lynch et al. 2008; Carlsen, Harling et al. 2013).

Within the United Kingdom, no study has examined the rate of return to work after colorectal cancer surgery and this is the first study to explore the impact of surgical technique upon return to work for colorectal cancer.

7.2 AIM

The aim of the present study was to assess return to normal holistic function at 1 year post potentially curative colorectal cancer resection within a single teaching hospital.

7.2 METHODS

7.2.1 Decisions for type of surgery offered

Within the time-frame of this study, some of our surgeons did not perform laparoscopic surgery. Patients were randomly allocated to a surgeon from a colorectal cancer multidisciplinary team meeting. Laparoscopically trained colorectal surgeons made choices regarding the type of surgery offered by a case-by-case basis encompassing patient preferences, individual patient factors and surgical experience.

7.2.2 Compiling a colorectal cancer resection database

A database of all colorectal resections was created by retrieving clinical details from our institution's electronic theatre system (ORMIS™) between 1 January 2011 and 1 June 2012 in order to facilitate a 1-year follow- up period. The iSOFT Clinical Manager system™ was used to cross reference each patient in order to check that the histological specimen matched theatre records and confirmed that the resection was for colorectal adenocarcinoma. This software was also used to check whether each patient was alive and to obtain a home address. Inclusion/exclusion criteria are shown in table 7.1.

Inclusion	Exclusion
Primary operation for colorectal adenocarcinoma	Disease recurrence
No stoma at 1 year post resection	
No ongoing oncological treatment at 1 year post resection	
Not deceased	

Table 7.1. Inclusion/exclusion criteria

7.2.3 Creating the questionnaire and contacting potential patients

A patient questionnaire was created in an electronic format using SnapSurveys™ software (Snap Surveys Ltd, London, UK) in consultation with, and approved by, the Risk and Clinical Governance Department at our institution (see appendix III). The questionnaire covered the following domains; surgical technique used, readiness at discharge, recovery expectations, time-frame for full recovery and information regarding pre- and postoperative employment. Each living patient who had undergone a colorectal resection was sent a cover letter describing the study, a questionnaire and a self-addressed pre-paid envelope for return.

7.3 RESULTS

Figure 7.1 demonstrates filtering of all operations performed within the time period of the study to identify eligible patients. Questionnaires were sent to 204 patients, with an overall response rate of 75% (n=153). Eighty- four percent (n=129 of 157) of responders had undergone open surgery and 16% (n=24 of

47) had had a laparoscopic procedure. Table 7.2 summarises the responses to perioperative questions and recovery. There was no statistical difference in patient perceived readiness for discharge and patient perceived adequacy of analgesia at discharge between open and laparoscopic groups. Reported recovery from laparoscopic surgery was significantly 'better than expected' compared to open surgery (Mann-Whitney U-test, $p<0.01$) and patients who had undergone laparoscopic surgery reported a significantly earlier 'return to full-fitness' than the open surgery group (Mann- Whitney U-test, $p<0.05$).

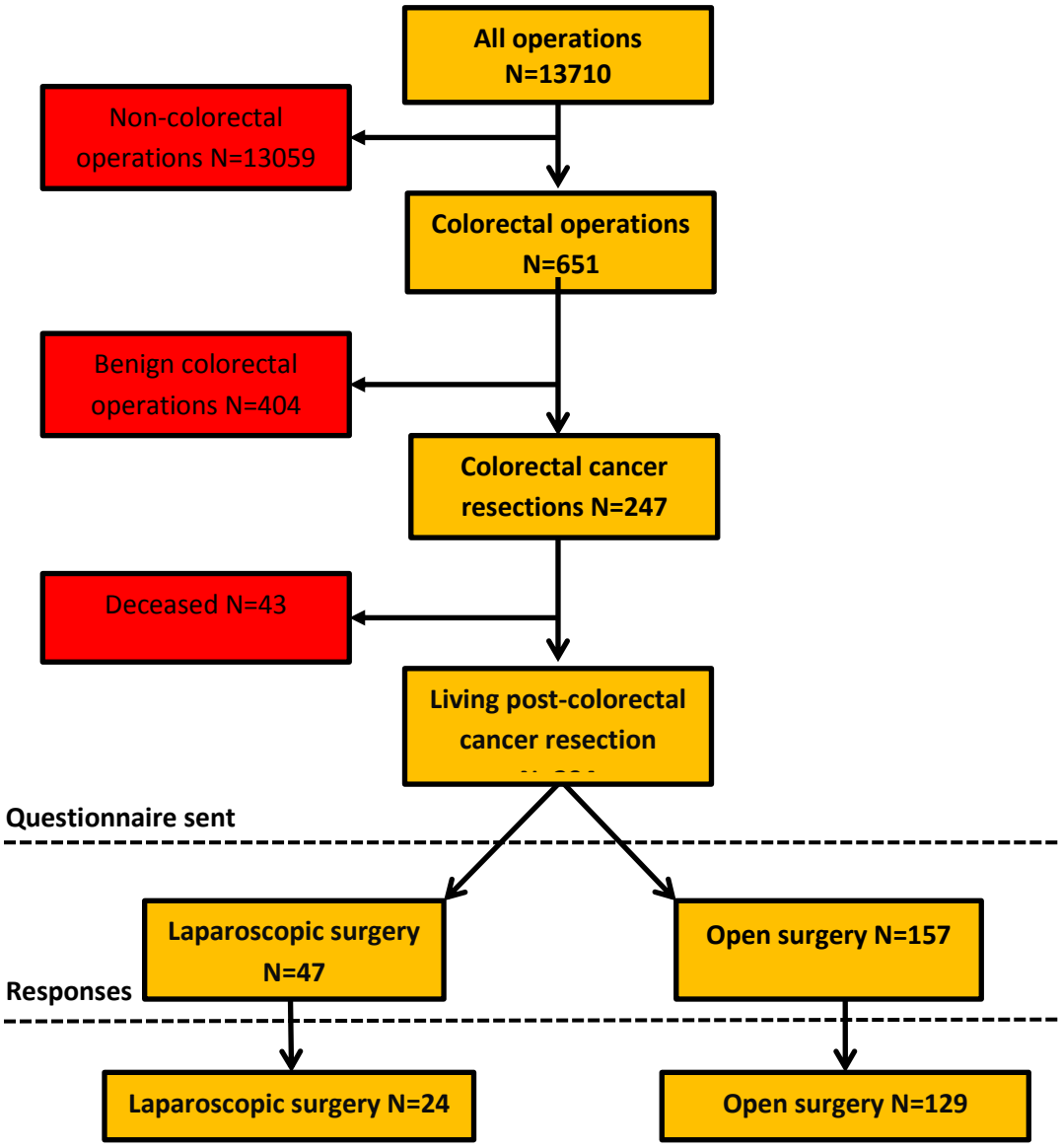


Figure 7.1 Flow chart for identification of eligible patients

	Operative technique		Statistical significance
	Open (n=129)	Laparoscopic (n=24)	
Median (range) age at operation	68 (48-91)	67 (36-84)	
Number of patients who felt ready when discharged	107 (83%)	20 (83%)	p=0.83 (Chi-squared)
Number of patients discharged with adequate analgesia	120 (93%)	22 (92%)	p=0.14 (Chi-squared)
Length of time taken to return to full fitness			p<0.05* (Mann-Whitney U test)
2-4 weeks	4 (3%)	4 (17%)	
1-3 months	21 (16%)	6 (25%)	
3-6 months	41 (32%)	7 (29%)	
>6 months	56 (43%)	7 (29%)	
Expectations for recovery			p<0.01* (Mann-Whitney U test)
A: Better than expected	33 (26%)	14 (58%)	
B: As expected	36 (28%)	5 (21%)	
C: Worse than expected	62 (48%)	5 (21%)	

Table 7.2 Responses to questions regarding age at surgery, adequacy of analgesia and recovery

** denotes statistically significant differences between open and laparoscopic surgery groups*

Employment status of responders before and after surgery is shown in table 7.3. There was no statistical difference between rates of employment prior to surgery between the groups (p=0.11). Return to work at 1 year was significantly more frequent after laparoscopic surgery (p<0.05) and length of time to return to work was significantly less after laparoscopic surgery (44(6-84) days) than open surgery (71(14-252) days)(p<0.05). Levels of self-employment were equal between the groups. Within the open surgery group, reasons for not returning to work after 1 year included voluntary retirement (n=10 of 18) and physical incapability (n=8 of 18).

	Operative technique	
	Open	Laparoscopic
Number of patients employed preoperatively	38	11
Number of patients returning to work	20	10*
Reasons for not working (number; median age)	Retirement (n=10; 62) Incomplete recovery (n=8; 58)	Retirement (n=1; 62)
Number of patients self-employed	17	4
Mean return to work (days)	71 (14-252)	44 (6-84)#

Table 7.3 Employment status of responders before and 1-year after surgery by open and laparoscopic approaches

**denotes statistically significant differences between open and laparoscopic surgery (p<0.05, Chi-square test); #denoted statistically significant differences between open and laparoscopic surgery (p<0.05, independent t-test)*

7.5 DISCUSSION

This is one of the first reports to simultaneously review subjective and objective aspects of recovery after colorectal cancer surgery and compare open with laparoscopic techniques. This study shows that 39% of employed patients diagnosed with colorectal cancer had not returned to work 1 year after surgery. Laparoscopic colorectal cancer surgery was associated with a significantly quicker return to full fitness, shorter delay until re-employment and an improved rate of return to work during the first year.

One of the most important factors governing recovery, especially return to work is age at the time of surgery. We found no statistical difference in median patient age between each group, and our median ages compare favourably with British national cancer incidence statistics (ONS 2008a).

Return to work was lower in our study compared with others, where up to 89% of patients had resumed work (Sanchez, Richardson et al. 2004). Reasons for this difference may be in the interpretation of the definition of 'return to work'. Some define return to work as return to full-time employment whilst others interpret this as employment of any type, including part-time or amended duties (Sanchez, Richardson et al. 2004; Taskila, Martikainen et al. 2007). We deliberately chose to use the phrase return to work in order to obtain information for the number of patients working in any capacity.

Patients with colorectal cancer are less likely to return to work compared with patients diagnosed with testicular, breast, endocrine or skin cancer (Amir, Moran et al. 2007; Carlsen, Dalton et al. 2008; Earle, Chretien et al. 2010). This may

be due to population-specific demographics associated with each cancer or differences in the relative morbidity associated with treatments. The association between disease-related factors and return to work after a cancer diagnosis including colorectal cancer has been observed in several studies where tumour stage, physical symptoms and American Society of Anesthesiologists score were all reported to be associated with a reduction in rates of return to work (Mols, Thong et al. 2009; Earle, Chretien et al. 2010; Gordon, Lynch et al. 2011).

Potential explanations for our low re-employment rate could also include variations in stoma creation/reversal rates, use of postoperative oncological therapies and state-funded social support offered by different countries. A recent study has reported that patients diagnosed with rectal cancer have significantly increased risk of sickness absence and early retirement due to either the presence of a stoma or from the increased prevalence of abnormal bowel habits or urinary function after surgery (Rodriguez-Bigas, Chang et al. 2007). Within the United Kingdom use and make up of chemotherapy regimens is uniform as the clinical decisions are made by multidisciplinary teams adhering to national guidelines. The effect of state-funded social support and/or public sector employment should not be underestimated. United Kingdom public sector employees (almost 19% of the work force) are entitled to additional 'sick-pay' benefits (ONS 2013). The high proportion of the population employed by the public sector combined with extensive social services support most likely has an impact upon the relatively low re-employment rates seen in this study. Sanchez et al noted up to 89% of patients returned to work after colorectal cancer treatment in the US (Sanchez, Richardson et al. 2004). Although differences exist in treatments offered to colorectal patients between various countries we should not underestimate the importance of patient motivation when comparing return to work figures. Patients in the UK may well be less motivated to return

to work due to the state-funded welfare-support offered by the government. Our questionnaire did not include specific questions focussed on public sector employment, ongoing postoperative symptoms, and the exact site and stage of the original tumour.

Although our response rate for patients who had had laparoscopic surgery was low, and the preoperative employment rate greater in the laparoscopic surgery group, this difference was not significant. However postoperative return to work at one year was significantly higher in patients who had undergone laparoscopic surgery. Reasons for not returning to work revealed that 10 of the 18 open patients decided to retire after surgery whilst 8 still had the intent to work in the future but were physically incapable in returning to work at the time of this questionnaire. Reasons given included, feeling 'weak', 'lethargic' or inability to physically contribute to 'heavy lifting'.

Patients who had undergone laparoscopic surgery also felt that they returned to full fitness significantly earlier than the open surgery group. The commonest response was a return to full fitness within 1-3 months for laparoscopic patients compared with more than 6 months most commonly reported in the open group. Interestingly, the laparoscopic group perceived their recovery to be significantly 'better than expected' whilst the open group reported recovery was 'worse than expected'. Clinical staff may under estimate the level of morbidity associated with an open operation. A recent study focusing on postoperative recovery for colorectal cancer patients from the United Kingdom reported that there was no consensus of opinion regarding either return to work or time-frame for recovery between members of the multidisciplinary team (Bains, Yarker et al. 2012). We found length of time for recuperation prior to return to employment significantly

reduced in patients having undergone laparoscopic surgery. This is unsurprising considering open surgery is associated with larger wounds producing greater morbidity which requires potentially stronger analgesia for a longer length of time. The mean length of time taken before return to work was 44 and 71 days for laparoscopic and open surgery respectively. These figures are similar to published consensus-based surgical guidelines of up to 6 weeks for laparoscopic surgery and between 6-10 weeks for open surgery (Palmer KT 2007).

Recent trends have focused on improving postoperative recovery include aspects of psychological well-being (Johnson M 1993). Our study noted the vast majority of patients felt 'ready for discharge', regardless of whether they underwent open or laparoscopic operations (even though these were at different times following surgery), indicating that communication was good and expectations for discharge were well set. Persistent pain because of inadequate analgesia may also prolong recovery via physiological and psychological effects. The majority of patients in our study reported access to adequate analgesia prior to discharge regardless of surgical technique.

Strengths of our study include the high response rate to the questionnaire, and that every participant followed the same perioperative enhanced recovery program, including aspects pertaining to psychological counselling, analgesic control and postoperative physical therapy. This is noted in the responses collected for 'adequacy of analgesia' upon discharge and 'readiness' when discharged, where there was no statistical difference between open and laparoscopic groups. The inclusion of self-employment rates is important as self-employed patients tend to be more motivated to return to work than patients working for others; however we found no correlation between self-employment

and return to work. We deliberately included both subjective and objective measures of recovery encompassing patient experiences, expectations and the time-taken for convalescence prior to return to work.

Our study does have limitations, with the potential for bias when reviewing the difference between the laparoscopic and open surgery groups. At the time of this study half of our surgeons performed laparoscopic surgery. Potential patients are randomly allocated to each surgeon from a colorectal cancer multidisciplinary team meeting. The laparoscopic surgeons attempt to perform a minimally invasive procedure unless patients had undergone previous complex abdominal surgery. This process is a potential source of bias, with more complex cases being manoeuvred into the open surgery group. We also had no information from responders regarding site of tumour, stage of disease and presence of a postoperative ileostomy. For this reason we decided to stipulate a one year follow up period allowing all patients adequate time to recover. We also noted a difference in response rates between laparoscopic and open surgery groups which may be due to the fact that patients undergoing laparoscopic surgery had resumed activities of daily living and had less time available to complete the questionnaire.

7.6 CONCLUSIONS

This is the first report in the United Kingdom of return to work after colorectal cancer surgery and was the first publication to review the impact of laparoscopic colorectal surgery on return to work. Over a third of patients had not returned to work 1 year after colorectal cancer surgery.

Regardless of the exact definition of postoperative recovery, patients having laparoscopic colorectal surgery returned to full fitness faster, felt recovery was shorter and returned to work quicker than those having open surgery. Large multicentre prospective studies are needed to fully understand the impact of potentially curative surgery for colorectal cancer. We must invest more in managing expectations and providing better post-discharge support (particularly to those undergoing open surgery) to improve recovery and return to work.

Chapter 8

General discussion & integrated themes

Improvements in healthcare and lifestyle have brought greater life expectancy leading governments to increase the age of retirement (Guardian 2014). Colorectal cancer is a common cancer in the United Kingdom, becoming more prevalent each year with the only proven cure being surgery (CRUK 2009). As a result more people diagnosed with colorectal cancer will require surgery in the future and a significant proportion will either need to return to work or resume normal daily activities in order to maintain independence.

Over the last twenty years the use of specific enhanced recovery programs has markedly improved recovery after colorectal cancer surgery, specifically the ability to discharge patients earlier (Aahlin, von Meyenfeldt et al. 2014). The most important facet responsible for this is the use of minimally invasive techniques. Technological advances have allowed surgeons to become bolder in the decisions made during surgery without compromising patient safety. The use of laparoscopic surgery is now the default position for colorectal cancer resection.

Shorter stay in hospital has led to the majority of postoperative functional recovery occurring after discharge. Data for long term functional recovery is limited. We present data from the first study to assess return to work after colorectal cancer surgery in the United Kingdom and the first publication to review the impact of laparoscopic colorectal surgery on return to work (Bhalla, Williams et al. 2014). Almost one third of patients who are of working age at the time of surgery failed to return to full-time employment with laparoscopic surgery associated with an increased rate of return to work in a significantly shorter time frame than open colorectal surgery.

Open techniques still have an important role in colorectal cancer resection as a significant proportion of patients are unable to undergo laparoscopic surgery. Potential factors associated with inability to perform laparoscopic surgery include, technical factors (ie. intra-abdominal adhesions after previous abdominal surgery or poor visualisation of structures due to lack of working space) and medical factors. Medical factors include increased body habitus, cardiac and respiratory diseases which potentially make insufflation and positioning hazardous and may have detrimental downstream effects upon inpatient recovery.

To improve long term functionality after colorectal cancer surgery we must understand and systematically analyse the entire journey patients undertake towards full recovery. General consensus amongst surgeons is that three periods of recovery exist namely, the immediate, early and long-term phases (Lee, Tran et al. 2014). The immediate phase accounts for time taken to recover from anaesthesia, whilst the early period corresponds to convalescence in hospital. The final phase is the time taken after discharge toward a full recovery.

Although studies have reviewed functional recovery, the majority assess the early postoperative period only, usually within the first month of recovery. In order to full assess a patient's journey toward full recovery more focus must be placed upon assessing patients with greater regularity and for considerably longer. No single study has specifically separated open and laparoscopic patients to review their individual recovery pathways.

This thesis was uniquely designed with the aim of facilitating an understanding of the processes taking place during the immediate, early and longer-term phases of recovery. Only after understanding these processes can novel interventions be planned for and placed appropriately to further improve functionality. Appropriate intervention has the potential to impact each individual and society in general, by improving independence and improving the socio-economic bourdon associated with inability to performing the activities of daily living.

One of the principle factors governing ability to conduct daily activities is strength. This is particularly important in an older population where small decreases in strength potentially have a significant impact upon morbidity and mortality. Minimal decreases in muscle mass have dramatic effects upon strength therefore small decreases in muscle mass potentially have dramatic affects upon morbidity and independence. The SMMACC study was powered specifically for changes in postoperative strength for this very reason.

We present evidence for underlying inflammatory processes related to the presence of colon cancer prior to surgery. There was little difference in the SSR measured by GLR between open and laparoscopic surgery. Almost certainly this was due to the fact that maximal differences in the SSR between open and laparoscopic surgery probably occur 4-6 hours post surgery and our first recording was 24 hours after surgery. Surgical stress responses continue for least 4 weeks postoperatively but after six months inflammatory levels fall below preoperative values. We hypothesise this is due to the absence of cancer mediated inflammation.

To explore changes in body composition, in particular skeletal muscle we analysed muscle architecture. Visualising the muscle architecture of *vastus lateralis* with B-mode ultrasound during the postoperative period is also unique. Being one of the principle anti-gravitational muscles used repetitively when walking and standing; changes within its architecture yield important information regarding a subject's mobility and activity levels.

The SMACC study is the first to quantify and visualise architectural changes occurring at regular intervals immediately after colorectal cancer surgery and over the following 6 months. The thickness and pennation angle of *vastus lateralis* decreases more after open colonic cancer surgery than for laparoscopic surgery. Maximum decreases in muscle thickness occur at 6 weeks post surgery and equate to an 8% muscle mass loss for open surgery compared to 2% for laparoscopic surgery. A significant positive correlation exists between changes in vastus lateralis muscle thickness and pennation angle ($r=0.69$, $p<0.05$).

The study potentially highlights how the technique of muscle architecture assessment with B-mode ultrasound may be moved from a research setting into a clinical one. Regular assessments may reveal patients at risk of excessive muscle mass losses in the early postoperative period (2-6 weeks) and additional support/advice could be initiated. It may then be possible to offset poor/delayed postoperative functional recovery. B-mode ultrasound has been shown to be a simple, reliable tool amenable to the outpatient setting making it a perfect diagnostic tool. Image capture of *vastus lateralis* together and measurement of muscle thickness takes only a matter of minutes. Indeed colorectal specialist nurses could be taught how to capture high quality images within a matter of hours allowing patients to be assessed in clinics. If combined with questionnaires

or hand-grip dynamometer readings a truly global assessment of early postoperative recovery is easily achievable.

The feasibility study using deuterium bio-labelling to quantify MPS rates over the first 6 weeks of recovery aimed to investigate the mechanisms contributing to loss of muscle mass. We are the first group to successfully perform a deuterium bio-labelling study within patients requiring surgery. Analysing the MPS rate over a period of 42 days is the longest assessment of the postoperative period ever reported. We note a MPS rate of 1.02±0.2%/day. This study opens the door for further rigorous assessment of muscle modulation within the postoperative period using the D_2O bio-labelling method. We plan to use the data from this study to provide information for future power calculations. Larger studies will aim to comparing MPS rates of patients having open and laparoscopic surgery. We will be able to vary the postoperative 'period of study' within each group by taking biopsies across various time points, allowing comparison of MPS rates between the groups at various phases during recovery.

Clear correlations are noted between several of the parameters measured during the SMMAC study. Figure 8.1 depicts the data obtained from both groups for GLR and plots them against the same individuals muscle thickness results from B-mode ultrasound assessment. A clear negative Pearsons correlation-product coefficient is seen confirming the hypothesis that inflammatory mediators may drive these catabolic processes ($r=-0.35$, $p<0.05$).

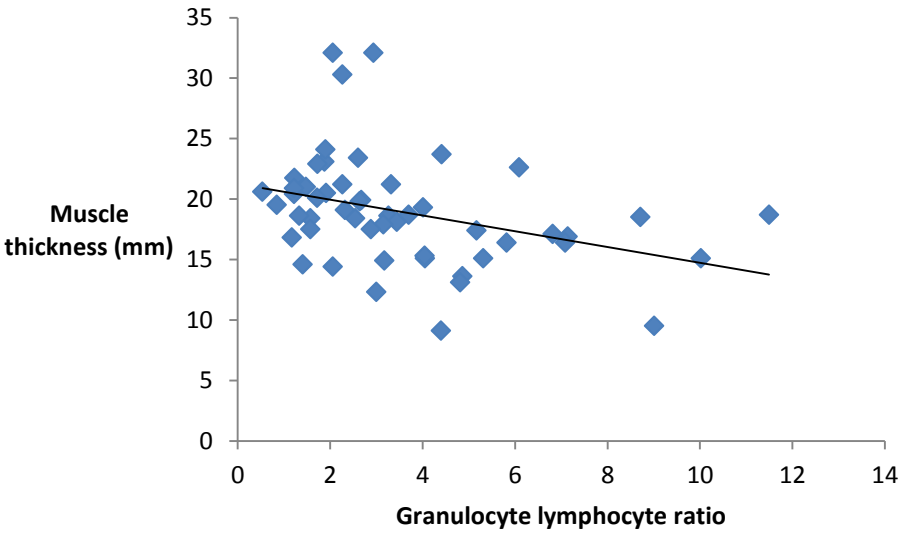


Figure 8.1 Correlation between muscle thickness and granulocyte lymphocyte ratio

Each blue dot represents one patient's results. The black line is the line of best fit (r=-0.35, p>0.05).

Figure 8.2 takes this hypothesis further by plotting the change in muscle thickness against strength. A positive Pearsons correlation coefficient is noted (r=0.34, p<0.01) providing further evidence for loss of muscle mass contributing directly to loss of strength. As stated earlier in this thesis it is well documented that minimal changes in muscle mass can produce marked changes in strength, especially in an elderly population.

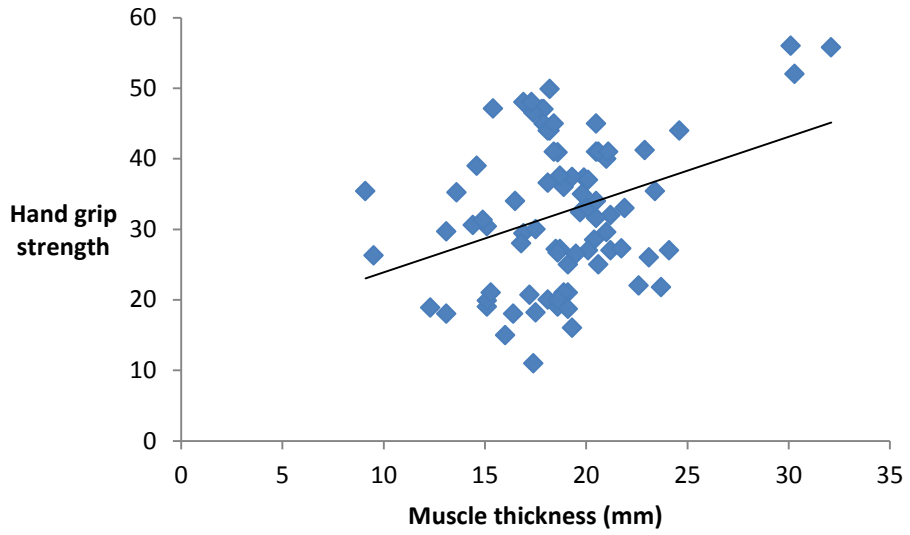
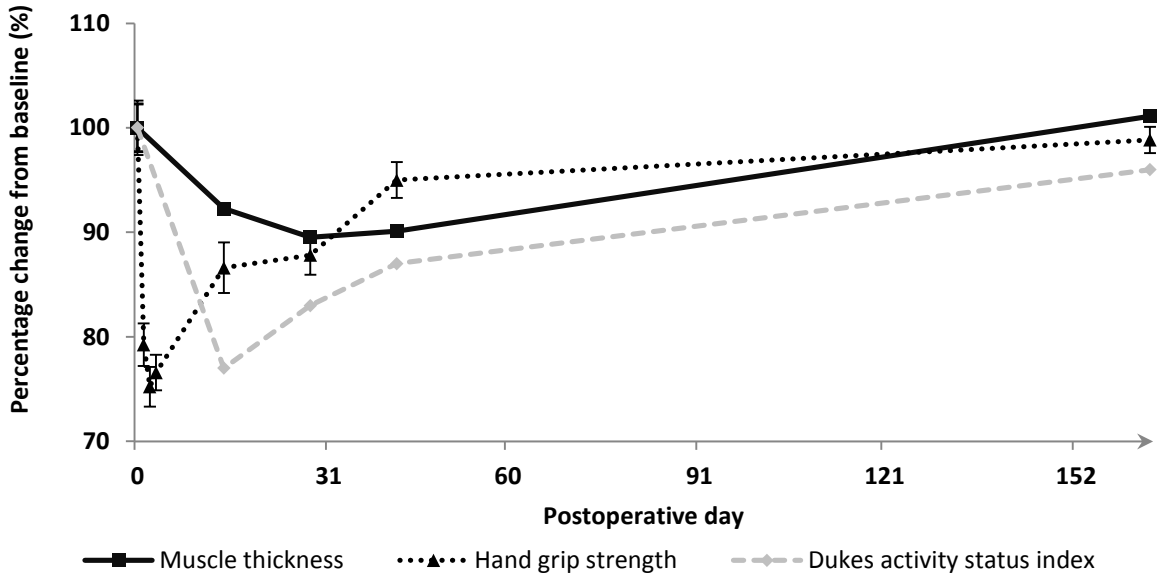


Figure 8.2 Correlation between muscle thickness and hand grip strength

Each blue dot represents one patient's results. The black line is the line of best fit (r=0.34, p>0.01).

An interesting aspect of recovery is the striking difference between patient reported functional outcomes and objective measurements of functionality/physical performance. We are the first group to map objective and subjective measures of functional outcome (figure 8.3). Looking closely at the graphs the disparity between objective and subjective measures is greater for the open surgery group. A possible explanation is that there may be important psychological aspects to recovery limiting or inhibiting patients from completing normal activities of daily living (Shehmar and Gupta 2010). This appears to be more pronounced in patients undergoing open procedures. This correlates with our data from EQ5d5l, where a proportion of patients (20%) described a degree of anxiety or depression prior to surgery. Psychological aspects may be equally as important to completing a full functional recovery as physical ones.

A



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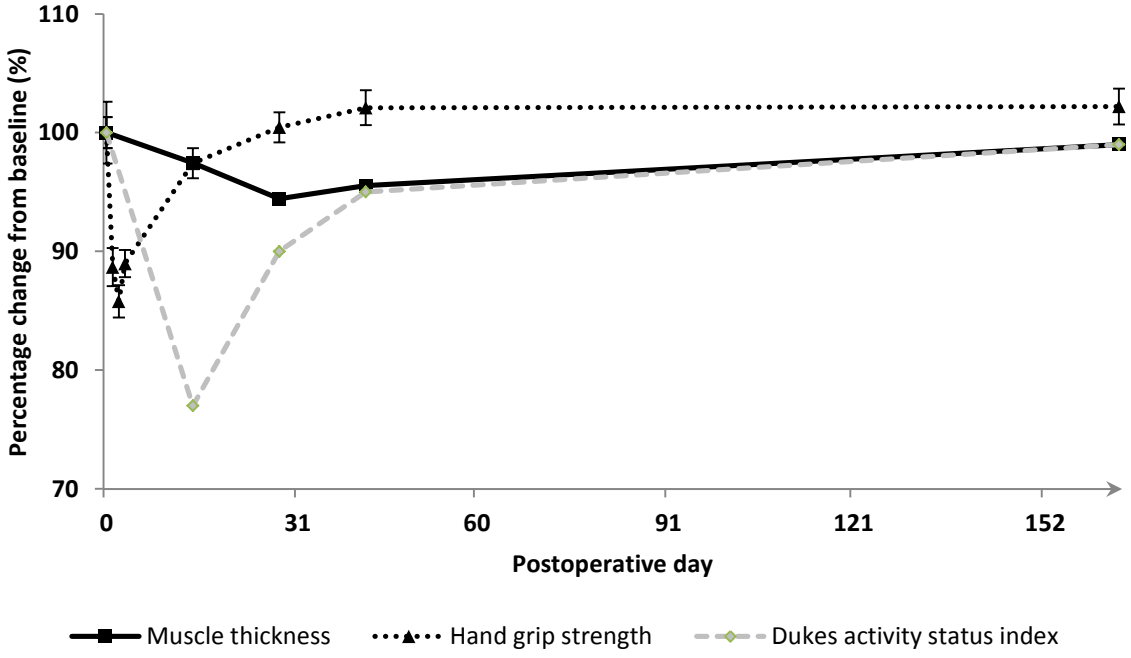

Figure 8.3 Timeline highlighting skeletal muscle modulation and functional recovery after colon cancer surgery

A- Open surgery, B- laparoscopic surgery

The psychological effect of being diagnosed with cancer and undergoing major surgery should not be overlooked. The term 'catastrophisation' has been used to explain this phenomenon. Catastrophisation was first coined by American psychologist Ellis in 1962 to describe a maladaptive cognitive style originally seen in patients with anxiety and depressive disorders with an irrational negative forecast of future events (Ellis 1971).

Catastophisation is potentially worse for patients undergoing a laparotomy either due to constant visualisation of the long scar or via positive reinforcement from health care providers (ie patients with laparotomy wounds must not undertake strenuous activities in order to protect the wound from possible incisional hernia).

Pain is a common negative experience which often signifies injury, illness, danger or possible doom. Combination of a cancer diagnosis, postoperative pain, a large scar and fear of complications may account for an increased incidence of psychological pressure upon patients undergoing open colorectal surgery.

Recent clinical studies review the effects of catastophisation upon physical disability (Noiseux, Callaghan et al. 2014; Vranceanu, Bachoura et al. 2014; Roh, Noh et al. 2015). The vast majority relate to patients undergoing orthopaedic or spinal surgery. Although methodology and results vary general consensus appears to note that poor coping strategies and irrational fear of damaging the site of surgery and/or fear of producing pain plays a part in limiting patient mobility/flexibility after surgery.

A study reviewing recovery after pelvic laparoscopy also highlighted poorer outcomes and pain profiles in patients with preoperative catastrophizing features (Jarrell, Ross et al. 2014). These irrational fears almost certainly exist in patients undergoing colorectal cancer surgery and at present modern enhanced recovery programs do not account for this phenomenon. Questionnaires may be able to profile patients coping mechanisms and predict catastrophisation. It may be beneficial for patients diagnosed with colorectal cancer to undergo assessment to address some of these problems prior to surgery.

In the absence of complications, the postsurgical period is associated with 20 to 40% reduction in physiological and functional capacity that may or may not return to preoperative levels, indeed we present evidence that one third of patients fail to return to work 1 year post colorectal cancer surgery (Bhalla, Williams et al. 2014).

Taking these findings into account, any intervention aimed at improving functional recovery and independence must focus upon resisting fatigue and muscle mass losses. Enhanced recovery programs have focussed upon reducing the SSR however there may be alternative avenues for potential intervention. Several groups have attempted to reverse excessive postoperative muscle modulation by building muscle in the postoperative period. These 'rehabilitiation' interventions have limited success (Gillis, Li et al. 2014). The work presented within this thesis highlights several problems with attempting to intervene within the postoperative period. We have shown cancer patients may be fatigued, suffering from pain, and may suffer psychological stress/anxiety regarding healing processes within the first 6 weeks post surgery. These factors severely limit the degree to which patients will participate in rehabilitation programs.

The preoperative period may be a more appropriate time to intervene. Poor preoperative physical performance increases risk of mortality, number of postoperative complications and prolongs functional recovery (Wilson, Davies et al. 2010; Lee, Tran et al. 2014). Patients are generally in a better physical condition preoperatively compared with the immediate and early postoperative period, and many have a prolonged wait between diagnosis and surgery. The process of enhancing an individual's functional capacity prior to scheduled surgery, aimed at improving the patient's tolerance to upcoming physiologic stress, has been coined 'prehabilitation' (Gillis, Li et al. 2014).

During the course of this thesis, a group in Canada performed a small parallel-arm single blinded superiority randomized controlled trial to compare the impact of a trimodal program initiated 4 weeks before surgery (prehabilitation) to an identical program (rehabilitation) initiated after surgery (Gillis, Li et al. 2014). The trial noted that the average 25.2m increase in preoperative walking capacity achieved with trimodal prehabilitation offset the average 21.8 meters decline observed with rehabilitation in the first 4 weeks after surgery; providing a buffer and facilitating a faster return to baseline walking capacity (Gillis, Li et al. 2014).

The majority of interventions (patients consuming high protein diets, undertaking aerobic and resistance training regimes and stress/anxiety relieving strategies) occurred unsupervised at each patient's home. Compliance was assessed with self-reported questionnaires and importantly greater compliance was noted in the prehabilitation group. No assessment of inflammation or body composition was undertaken and it is unclear which particular component of the trimodal intervention contributed most to recovery. It is also unclear whether

improvements in functional walking capacity translate to clinically relevant outcomes.

An observation within this thesis is that strength and functionality are diminished to a greater extent than the loss of muscle mass. Other factors must be involved. A considerable proportion of patients (20%) reported a degree of anxiety or depression prior to surgery. As highlighted earlier (figure 8.3) a proportion of the reduction in functionality may have a psychological basis (ie. catastrophisation). Theoretically if this psychological stress is combined with a degree of sarcopenia and/or cancer cachexia patients will struggle to regain full functional capacity within the first few months after surgery.

The knowledge and expertise gained from this thesis provides novel methods of assessing the SSR and muscle modulation. These may aid further assessment of the postoperative period. We have shown that the SSR can be assessed quickly at minimal cost using GLR and that B-mode ultrasound can assess changes in body composition relating to muscle mass reliably and easily at any stage in the pre and postoperative period. Our preliminary work with D_2O bio-labelling potentially allows assessment of MPS rates either at specific time points or across the entire length of the postoperative period.

We would like to use the knowledge gained from this thesis to introduce our own trimodal prehabilitation program. Regarding the physical component of a potential trimodal prehabilitation program; preoperative high intensity training regimes may improve long tern functionality. A recent pilot study conducted in conjunction with our muscle physiology department has noted an improved

anaerobic threshold in patients over the age of 60 within a 3 week exercise program. This form of training is an ideal model for prehabilitation by encouraging participation in short but vigorous exercise programs in the weeks after diagnosis leading up to surgery. Anxiety, depression and catastrophisation may be predicted preoperatively by formal assessment. Should any issues be highlighted, intervention with a trained psychotherapist providing group based cognitive behaviour therapy could be instigated. Individuals with similar problems may benefit from interacting with one another. We plan to conduct a pilot study to attempt to predict patients who may be at risk of suffering from catastrophisation with view to intervention. Identifying anxiety or personality traits associated with catastrophisation and improving muscle modulation through exercise is important but nutritional supplementation with protein/carbohydrate supplement may further offset changes associated with cancer cachexia and sacropenia. Use of B-mode ultrasound and D_2O techniques would allow direct assessment of muscle modulation.

This thesis adds to growing knowledge regarding the effects of colorectal cancer surgery on postoperative functional ability. We use modern techniques to investigate the effects of the SSR and muscle modulation offering insight to the serological and morphological adaptations that occur. We attempt to relate these changes to postoperative functionality over a 6 month period. We highlight new avenues of research where postoperative functionality may be improved for a large proportion of patients with the potential to benefit individuals and society in general.

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Appendix

Section I: Ethical approval documentation

The University of Nottingham

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20th December 2012

Dear Sir or Madam,

Sponsorship Statement

Re: Skeletal muscle modulation after open and minimally invasive surgery for colorectal adenocarcinoma

I can confirm that this research proposal has been discussed with the Chief Investigator and agreement to sponsor the research is in place.

An appropriate process of scientific critique has demonstrated that this research proposal is worthwhile and of high scientific quality.*

Any necessary indemnity or insurance arrangements will be in place before this research starts. Arrangements will be in place before the study starts for the research team to access resources and support to deliver the research as proposed.

Arrangements to allocate responsibilities for the management, monitoring and reporting of the research will be in place before the research starts.

The duties of sponsors set out in the NHS Research Governance Framework for Health and Social Care will be undertaken in relation to this research.**

* Not applicable to student research (except doctoral research).
** Not applicable to research outside the scope of the Research Governance Framework.

Yours faithfully

Angela Shone
Head of Research Governance
University of Nottingham

world-changing research
from The University of Nottingham

Derby Hospitals NHS
NHS Foundation Trust

Royal Derby Hospital
Uttoxeter Road
Derby
DE22 3NE

Tel: 01332 340131
Minicom: 01332 254944
www.derbyhospitals.nhs.uk

Research and Development Office

TRUST APPROVAL LETTER

Dr John Williams
Associate Professor and Honorary Consultant of Anaesthesia
School of Graduate Entry Medicine and Health
Royal Derby Hospital
Uttoxeter Road
Derby DE22 3DT

Dear Dr Williams

Re: Skeletal muscle modulation after open and minimally invasive surgery for colorectal adenocarcinoma

R&D Ref: DHRD/2013/016

Further to the Research Ethics Committee approval for the above study, I am pleased to confirm Trust management approval for you to proceed in accordance with the agreed protocol, the Trust's financial procedures for research and development and the Research Governance Framework (which includes the Data Protection Act 1998 and the Health & Safety at Work Act 1974).

Please supply the following to Dr Teresa Grieve, Assistant Director of R&D:

- the actual start and end dates of this study (**before the study commences**).
- details of any publications arising from this research project.
- a final report and a report every six months if the study duration is greater than six months.
- notification of any SUSARS, amendments, urgent safety measures or if the trial is abandoned.

Please note that approval for this study is dependent on full compliance with all of the above conditions.

I would like to take this opportunity to wish you every success with this study.

Yours sincerely,

Teresa M Grieve

P.P.

Prof. Richard Donnelly MD, PhD, FRCP, FRACP
Director of Research & Development

NHS
Health Research Authority

NRES Committee East Midlands - Nottingham 1
The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

Telephone: 0115 8839368
Facsimile: 0115 8839294

05 February 2013

Dr John Williams
Associate Professor
University of Nottingham
School of Graduate Entry Medicine and Health
Royal Derby Hospital
Uttoxeter Road
DE22 3DT

Dear Dr Williams

Study title:	**Skeletal muscle modulation after open and minimally invasive surgery for colorectal adenocarcinoma**
REC reference:	**13/EM/0031**
Protocol number:	**12134**
IRAS project ID:	**122038**

Thank you for your letter of 24 January 2013, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the Co-ordinator Mrs Lisa Gregory, nrescommittee.eastmidlands-nottingham1@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see

"Conditions of the favourable opinion" below).

Non-NHS sites

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Covering Letter		24 December 2012
Evidence of insurance or indemnity		06 August 2012
Investigator CV		07 December 2012
Investigator CV	Mr Ashish Bhalla	08 December 2012
Letter from Sponsor		20 December 2012
Other: MHRA Confirmation that CTA is not required		11 October 2012
Other: GP Information Letter	1.0	17 December 2012
Other: Numerical Rating Pain Scale		
Other: Data Sheet - Deuterium Oxide (Heavy Water)	1.1	24 January 2013
Participant Consent Form	1.0	17 December 2012
Participant Information Sheet	1.1	24 January 2013
Protocol	Final Version 1.0	17 December 2012
Questionnaire: EQ-5D-5L		

Questionnaire: DASI		
REC application	122038/397205/1/317	21 December 2012
Response to Request for Further Information		24 January 2013
Summary/Synopsis	1.0	17 December 2012

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

13/EM/0031	**Please quote this number on all correspondence**

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

Yours sincerely

Mr Robert Johnson
Chair
Email:nrescommittee.eastmidlands-nottingham1@nhs.net

Appendix

Section II: Patient information

DERBY HOSPITALS NHS FOUNDATION TRUST
ERAS PATHWAY

Pre-Operative Assessment
Provide ERAS info & Anaesthetic Information
Provide Carbohydrate Drinks
Physiotherapy information provided

↓

Patient Admitted on Day of Surgery
2 x Carbohydrate Loading Drinks up to 2hours pre-op
Admitted to Elective Admissions Ward
Patient given pre-emptive analgesia - 2g Paracetamol
Administer all usual meds (except ACE inhibitors & Angiotensin II antagonists)
Anti-embolism stockings applied

↓

Surgery
Spinal with IT Diamorphine
Oesophageal Doppler Targeted Fluid Management
Prophylactic Anti-emetics
IV Ketorolac if no contra-indications
TAP block or surgical infiltration at end of procedure

↓

Post-Op - Patients go to ward 309 post-op (if no co-morbidities)
Drink fluids in recovery
Regular Paracetamol & NSAID (if no contra-indications) + Regular anti-emetic
IV fluids at 1ml/kg/hr to a max of 100mls/hr
PCAS
Allow soup/jelly/ice cream if desired
Encourage patient to chew gum
Stand & walk a few paces
Anti-embolism stockings in-situ

↓

DAY 1
Diet & Fluids as tolerated + Fortisips or Fortijuice
Administer Paracetamol, Tramadol & NSAIDs at 0800
Stop PCAS & IVI
Walk 30m twice
Sit out of bed for 6 hours throughout the day
Remove urinary catheter (unless otherwise stated)
Anti-embolism stockings & teach patient Enoxaparin administration
Encourage patient to chew gum
Discharge planning

↓

DAY 2
Diet & Fluids as tolerated + Fortisips or Fortijuice
Administer Paracetamol, Tramadol & NSAIDs
Walk 60m twice
Remove drains (unless otherwise stated)
Anti-embolism stockings & patient to administer Enoxaparin
Continue discharge planning

↓

DAY 3
Diet & Fluids + Fortisips or Fortijuice
Independent mobilisation
Provide VTE information and discharge home with Enoxaparin (28 days in total)
2/52 OPA with Colorectal Nurse

↓

Discharge home. District or Practice Nurse for ROS at day 10

The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

Participant Information Sheet
(Final version 1.1: 24/01/13)

Skeletal muscle changes after colorectal surgery
Name of Researcher(s): Dr John Williams, Mr Ashish Bhalla

We would like to invite you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. Talk to others about the study if you wish. Ask us if there is anything that is not clear.

What is the purpose of the study?
The aim of this study is to understand what changes occur in your muscle after you have surgery and what affect this has on you in order to improve things for people in the future. This study will also form part of a PhD that our research fellow is working towards.

Why have I been invited?
You are being invited to take part because you are undergoing an operation for colorectal cancer. We are inviting everyone undergoing operations for colorectal cancer to take part.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. This would not affect your legal rights.

What will happen to me if I take part?
You will undertake regular assessments for up 8 months. The flow chart attached summarises your actual involvement. Potential participants (patients with colorectal cancer offered an operation) will be highlighted from the Derbyshire NHS trusts colorectal multidisciplinary team meeting. You will be seen by a research fellow after your outpatient meeting with your surgeon. Information packs containing details of the study will be given to you and any questions answered. You will be given the packs to take away and then you can decide whether you would be keen to participate. If you are interested to take part you will be consented by the research fellow during your pre-operative clinic appointment.

At this time the following additional assessments will be conducted:
1. Muscle architecture study (Using an ultrasound scanner, a photograph of your thigh muscles (quadriceps) will be taken for analysis at a later date to review how it changes over time)

2. Strength tests (hand grip and leg extension (kicking) strength will be gauged using a simple bedside test and the changes reviewed over time)
3. Skin fold thickness will be estimated using a calliper on the back of the arm (triceps muscle) to assess fat percentage changes over time.
4. Three questionnaires will be filled out (EQ-5D quality of life; visual pain scale; DASI mobility/activity score).
5. 5-10mls (two teaspoons) Serum blood sample will be taken to review markers of inflammation over time.
6. Exercise tolerance will be tested with a 3 minute step-exercise test to gauge heart rate after this exercise

Upon discharge from this clinic you will be given 200mls of 'heavy' water (containing 95% deuterium oxide) to drink the day before surgery.

Operative assessments
During the operation, whilst you are asleep (under general anaesthesia) the operating surgeon will excise (remove) a 1x1cm biopsy of skeletal muscle from your wound which will be sent to our laboratory (in liquid nitrogen) for assessment.

Post Operative assessments
Post-operative day 1. You will be visited on the ward by the research fellow who will conduct the assessments 1-5 noted above (total time 15mins). You will also be asked for 2mls of saliva which will be collected and analysed in a laboratory for heavy water concentration levels.

Post-Operative day 2 until discharge you will undertake tests 1-3 on the ward with the research fellow (10mins). Again daily saliva samples will be collected as above.

Upon discharge you will be given 2 bottles of 200mls heavy water. One bottle will be consumed post-operative day 8 and the second at day 15 (research fellow will remind you on those particular days with a simple phone call). All patients will be given containers to collect saliva samples each day. You keep hold of these until your next assessment. Future assessments can either occur in hospital or the research fellow can attend your home (patients choice).

Post-operative day 15 and 29. You will undergo all assessments above 1-6 (either in the clinical research laboratory or at home). At this point saliva samples will be collected and 2 new bottles of 200mls heavy water and fresh containers for saliva samples given to you.

At post-operative day 43 you will attend our outpatient department where tests 1-6 will be performed. No further heavy water will be given and no further saliva collected. A muscle biopsy from the quadriceps muscle in the thigh will be performed under local anaesthetic. This involves numbing a patch of skin over you thigh, making a 2cm incision (cut) over the skin and taking a small sample of muscle underneath. The wound will be stitched closed

and a plaster placed over it. The stitches will be removed in 3-5 days by a nurse at your GP surgery or by the research fellow (your choice).

Lastly, you will then be seen at 6 months post-operatively for a final assessment (1-6 above) and any results obtained given directly to you. We will then thank-you before you leave the study.

Expenses and payments
Participants will not be paid to participate in the study.

What are the possible disadvantages and risks of taking part?
Some patients may feel nausea after taking the deuterium oxide for several weeks. Known side effects include nausea, vomiting and abdominal pain (cramps). Usually these symptoms occur when levels of deuterium oxide are above 20%. We will aim to keep levels at under 5% and will check levels by analysing the saliva samples taken from you. If you have any side effects at all we will cease giving it to you.

Side effects from the muscle biopsy include pain, bleeding and very rarely an infection. We will give you simple painkillers for the pain and antibiotics for any infections. We will not discharge you until we are satisfied that there is no bleeding.

What are the possible benefits of taking part?

We cannot promise the study will help you but the information we get from this study may help us understand how people recover after an operation and then we will be able to modify our outpatient treatments to help others like yourselves in the future.

What happens when the research study stops?
When the research ends we will thank you and let you know our results and what we have gained.

What if there is a problem?

If you have a concern about any aspect of this study, you should speak to us and we will do our best to answer your questions. Our contact details are given at the end of this information sheet. If you remain unhappy and wish to complain formally, you can do this by contacting the Patient Advice and Liaison services (PALS) at the Royal Derby Hospital (01332 787258).

Will my taking part in the study be kept confidential?
Yes. We will be meeting you at all your assessments and all the samples I take from you will be kept confidential. Your details will be placed into a file that will be stored in a locked cupboard within a locked office. An electronic backup will also be password protected. All analysis will occur in the university only. Once we have collected all the data, if you agree, we may keep some of the tissue samples collected for further studies.

We will follow ethical and legal practice and all information about you will be handled in confidence.

If you join the study, some parts of your medical records and the data collected for the study will be looked at by authorised persons from the University of Nottingham who are organising the research. They may also be looked at by authorised people to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and we will do our best to meet this duty.

All information which is collected about you during the course of the research will be kept **strictly confidential**, stored in a secure and locked office, and on a password protected database. Any information about you which leaves the hospital will have your name and address removed (anonymised) and a unique code will be used so that you cannot be recognised from it.

Your personal data (address, telephone number) will be kept for 6-12 months after the end of the study so that we are able to contact you about the findings of the study *and possible follow-up studies* (unless you advise us that you do not wish to be contacted). All other data (research data) will be kept securely for 7 years. After this time your data will be disposed of securely. During this time all precautions will be taken by all those involved to maintain your confidentiality, only members of the research team will have access to your personal data.

What will happen if I don't want to carry on with the study?
Your participation is voluntary and you are free to withdraw at any time, without giving any reason, and without your legal rights being affected. If you withdraw then the information collected so far cannot be erased and this information may still be used in the project analysis.

Involvement of the General Practitioner/Family doctor (GP)
We will be informing your GP about your participation in this study.

What will happen to any samples I give?
All samples (saliva, muscle and tumour) will be analysed by our team to look at how your body has reacted to the operation. They will be stored and analysed on site.

The saliva samples will be analysed for levels of heavy water, the muscle samples will be used to find out how much new muscle has been produced since the operation and the tumour samples will be analysed to estimate how quickly it was growing.

We would also like to seek your consent so that any remaining samples may be stored and used in possible future research – this is optional (please indicate you agree to this on the consent form). The samples will be stored with a code unique to you and securely at the

University of Nottingham under the University's Human Tissue Research Licence (no 12265).

Some of these future studies may be carried out by researchers other than our current team (Dr Williams) who ran the first study, including researchers working for commercial companies. Any samples or data used will be anonymised, and you will not be identified in anyway. If you do not agree to this any remaining samples will be disposed of in accordance with the Human Tissue Authority's codes of practice.

Will any genetic tests be done?
No

What will happen to the results of the research study?
We will write to each patient with the results/findings from this study. The results will be used as part of an educational thesis and published in a scientific journal. We may present our findings internationally.

Who is organising and funding the research?
This research is being organised by the University of Nottingham and funded by MRC/ARUK Centre for Musculoskeletal Ageing Research.

Who has reviewed the study?
All research in the NHS is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by the Nottingham(1) Research Ethics Committee.

Further information and contact details
Mr Ashish Bhalla
Clinical research fellow
Graduate Entry Medical School
University of Nottingham
Uttoxeter Road
DE22 3DT
Telephone: 01332 724641

CONSENT FORM
(Draft Version 1.3 / Final version 1.0: 17/12/12)

Title of Study: Skeletal muscle modulation after colorectal

REC ref: 13/EM/0031

Name of Researcher: Mr Ashish Bhalla

Name of Participant: **Please initial box**

1. I confirm that I have read and understand the information sheet version number 1.0 dated 17/12/12 for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected. I understand that should I withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis. ☐

3. I understand that relevant sections of my medical notes and data collected in the study may be looked at by authorised individuals from the University of Nottingham, the research group and regulatory authorities where it is relevant to my taking part in this study. I give permission for these individuals to have access to these records and to collect, store, analyse and publish information obtained from my participation in this study. I understand that my personal details will be kept confidential. ☐

4. I understand and agree that saliva, muscle and tumour samples will be taken for analysis of deuterium oxide levels, protein synthesis and DNA synthesis. ☐

5. Consent for storage and use in possible future research (Optional)
I agree that the samples I have given and the information gathered about me can be stored by John Williams at the Graduate entry medical school, for possible use in future studies. I understand that some of these studies may be carried out by researchers other than current team of clinical physiology unit, who ran the first study, including researchers working for commercial companies. Any samples or data used will be anonymised, and I will not be identified in anyway. ☐

6. I agree to my GP being informed of my participation in this study. ☐

7. I agree to take part in the above study. ☐

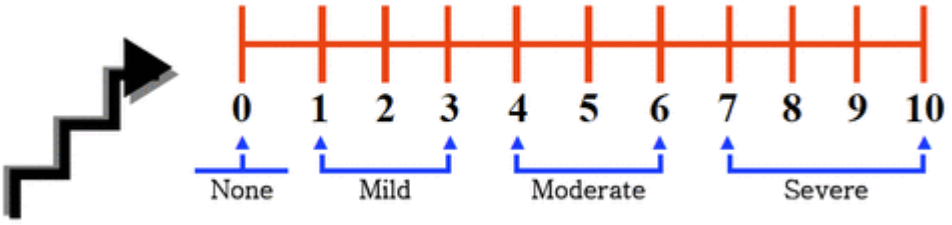
Name of Participant	Date	Signature
Name of Person taking consent	Date	Signature

3 copies: 1 for participant, 1 for the project notes and 1 for the medical notes

Appendix

Section III: Questionnaires

Numerical Rating Pain Scale



Rating Pain Level

0 No Pain

1 – 3 Mild Pain (nagging, annoying, interfering little with activities of daily living)

4 – 6 Moderate Pain (interferes significantly with activities of daily living)

7 – 10 Severe Pain (disabling; unable to perform activities of daily living)

Score ☐

Recruitment/identifier number:

Date:

Assessment:

Duke Activity Status Index (DASI)

Can you (circle yes or no):

1. Take care of yourself--that is, eat, dress, bathe, or use the toilet?	**Yes**	**No**	2.75
2. Walk indoors, such as around your house?	**Yes**	**No**	1.75
3. Walk a 200 yards on level ground?	**Yes**	**No**	2.75
4. Climb a flight of stairs or walk up a hill?	**Yes**	**No**	5.50
5. Run a short distance?	**Yes**	**No**	8.00
6. Do light work around the house like dusting or washing dishes?	**Yes**	**No**	2.70
7. Do moderate work around the house like vacuuming, sweeping floors, or carrying groceries?	**Yes**	**No**	3.50
8. Do heavy work around the house like scrubbing floors or lifting or moving heavy furniture?	**Yes**	**No**	8.00
9. Do yard work like raking leaves, weeding, or pushing a power mower?	**Yes**	**No**	4.50
10. Have sexual relations?	**Yes**	**No**	5.25
11. Participate in moderate recreational activities like golf, bowling, doubles tennis, or throwing a baseball or football?	**Yes**	**No**	6.00
12. Participate in strenuous sports like swimming, singles tennis, football, basketball, or skiing?	**Yes**	**No**	7.50

{DASI=sum of 'yes' replies, VO_2 peak (ml/kg/min) = (0.43xDASI) + 9.6}

Recruitment/identifier number:

Date:

Assessment:

(English version for the UK)

Under each heading, please tick the ONE box that best describes your health TODAY

MOBILITY

I have no problems in walking about ❑
I have slight problems in walking about ❑
I have moderate problems in walking about ❑
I have severe problems in walking about ❑
I am unable to walk about ❑

SELF-CARE

I have no problems washing or dressing myself ❑
I have slight problems washing or dressing myself ❑
I have moderate problems washing or dressing myself ❑
I have severe problems washing or dressing myself ❑
I am unable to wash or dress myself ❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

I have no problems doing my usual activities ❑
I have slight problems doing my usual activities ❑
I have moderate problems doing my usual activities ❑
I have severe problems doing my usual activities ❑
I am unable to do my usual activities ❑

PAIN / DISCOMFORT

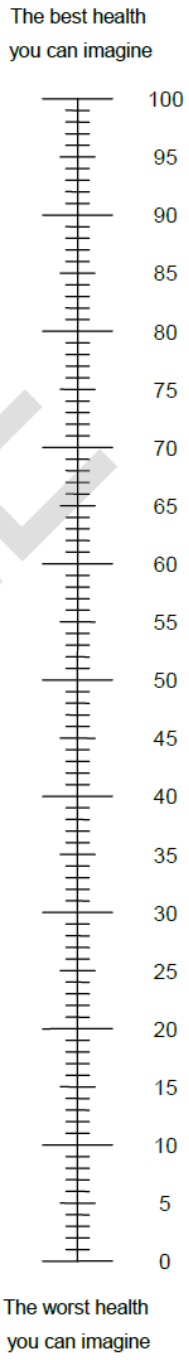
I have no pain or discomfort ❑
I have slight pain or discomfort ❑
I have moderate pain or discomfort ❑
I have severe pain or discomfort ❑
I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

I am not anxious or depressed ❑
I am slightly anxious or depressed ❑
I am moderately anxious or depressed ❑
I am severely anxious or depressed ❑
I am extremely anxious or depressed ❑

- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the <u>best</u> health you can imagine.
  0 means the <u>worst</u> health you can imagine.
- Mark an X on the scale to indicate how your health is TODAY.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



DERBY COLORECTAL UNIT- Return to work questionnaire

N Hurst PhD FRCS Consultant	01332 788762	J N Lund DM FRCS Ass Professor	01332 788762
J R Reynolds DM FRCS Consultant	01332 786469	W J Speake DM FRCS Consultant	01332 788758
G Tierney DM FRCS Consultant	01332 785590	A Hall Nurse Specialist	01332 787237
D Reeves Nurse Specialist	01332 787236	Stoma Care	01332 787465

Fax No: 01332786099

PLEASE CIRCLE YOUR ANSWERS

1. Did you have key-hole surgery (several small scars) or an open operation (one large scar)?
   Key-hole **Open** **Not sure**

2. What age were you when you had your operation?

3. Were you working before your operation?
   Yes **No**

   (a) If yes, did you go back to work or if you couldn't get back to work was there a particular reason?
   Yes **No** (any particular reason)

   (b) Were/are you self-employed?
   Yes **No**

   (c) How long after your operation did you return to work?

   (d) If yes, did you have to change your type of work or hours?
   Yes **No**

4. Did you feel ready for home when you were discharged?
   Yes **No**

5. Did you have adequate pain control after leaving the hospital?
   Yes **No**

6. How long after your operation did you feel back to full fitness?
   2-4 weeks **1-3 months** **3-6 months** **< 6 months**

7. Was your recovery…
   Better than you expected **As you expected** **Longer than you expected**

8. Please feel free to write any additional comments if you wish