# Non-invasive Approaches to Identify the Cause of Premature Fatigue in Inflammatory Bowel Disease Patients.


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**Abstract**

Inflammatory bowel disease (IBD) fatigue is a pervasive secondary disease symptom. The aetiology is poorly understood, meaning that treatment options are sparse. This is of particular concern for the relatively large proportion of patients with quiescent disease, who continue to report an increased perception of fatigue and demonstrate premature exercise fatigue, relative to healthy individuals. Fatigue is multidimensional and can manifest as a disproportionate perception of tiredness, perturbed cognitive functioning and an inability to sustain a required work output during exercise.

In contrast to other chronic disease, to date there has been no mechanistic assessment of IBD fatigue reported in the literature. This is congruent with the essential absence of any effective treatment strategies convincingly shown to reduce IBD fatigue burden, independent of targeting known clinical causes.

The application of Magnetic Resonance Imaging (MRI) and Spectroscopy (MRS) techniques during exercise represents a unique opportunity to non-invasively probe *in-vivo* metabolism across multiple organs. This thesis seeks to characterise IBD fatigue aetiology by combining laboratory-based assessment of peripheral muscle function and cardiorespiratory fitness, with proton ($^{1}$H) MRI and phosphorus ($^{31}$P) MRS during within-bore exercise.

This thesis represents the first attempt to comprehensively interrogate IBD physiology with the aim of identifying potential treatment targets for fatigue.

Following an introduction to IBD in Chapter one, a detailed review of IBD fatigue aetiology follows in Chapter 2. Chapters 3 and 4 outline the methodology and developmental experiments undertaken to facilitate the MRI and $^{31}$P MRS experiments. Chapter 5 details the assessment of peripheral muscle function and body composition in quiescent Crohn's disease

patients relative to a healthy age and BMI matched control group. This is followed by the assessment of cardiovascular, brain and peripheral muscle deconditioning in Chapter 6 and 7.

A final discussion chapter is dedicated to a review of the collective findings of this thesis in the context of existing data within the literature base. Suggestions are then made for future research priorities in the field of IBD fatigue.

**Contents**

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# Chapter 1: Inflammatory bowel disease (IBD)

## 1.0 Introduction

The purpose of this chapter is to provide the reader with an introduction into inflammatory bowel disease (IBD). Disease definition and epidemiology are outlined initially. The following sections provide information on the aetiology of IBD and factors with the disease. Finally, disease symptomology, methods of diagnosis and clinical management strategies are outlined. This brief overview outlines key information required for an understanding of IBD, prior to an in-depth review of IBD fatigue aetiology in chapter two.

## 1.1 Inflammatory bowel disease

## 1.2 Definition

Inflammatory bowel disease (IBD) primarily refers to the systemic inflammatory diseases Crohn's disease (CD) and Ulcerative Colitis (UC). Both conditions manifest in the gastrointestinal tract (GIT) and exhibit a chronic, relapsing nature [1]. Whilst UC is specific to the mucosa and submucosal layers of the colon, CD is transmural and can affect any area of the gastrointestinal tract (GIT) from the mouth to the anus [2]. UC was first characterised in 1909 [3] with CD established later as a separate disease entity in 1932 [4]. Despite the plethora of research carried out in IBD over the last eight decades, the aetiology of the disease remains poorly understood and there is currently no known cure.

## 1.3 Epidemiology

### 1.3.1 Incidence and distribution by age

The incidence of IBD varies greatly according to geographic location. IBD has classically displayed higher incidence rates in the western world and is associated with industrialisation. However, more recent data demonstrate an emerging trend of increased incidence in eastern countries [5], though annual incidence rates still remain significantly higher in the western world. For example, the annual incidence of CD in Canada is 20.2 per 100,000 [6] and 10.6 per 100,000 in the United Kingdom [7]. UC displays similar prevalence with 19.2 people per 100,000 in Canada [8] and 24.3 people per 100,000 in an Icelandic cohort [9] This is in stark contrast to IBD incidence rates of 0.76 for UC and 0.54 for CD per 100,000 in Asia-Pacific [10]. Whilst IBD can occur at any age, available epidemiological data demonstrates a peak age of onset for CD between 20 and 30 years of age [11]. Despite this, peaks in incidence in both CD and UC have been reported between the 7th and 8th decade of life in a Danish cohort [12]. The median age at CD diagnoses in North American cohorts has been reported at 29.5 years with a range of 4.2 – 83.8 years [13]. Whilst the median age of diagnoses for UC is of 34.5 years with a range of 7-90 years in a consistent geographical location [14].

### 1.3.2 Prevalence

Consistent with incidence rates, the prevalence of IBD is highest in Europe, with 505 per 100,000 of UC in Norway [15] and 322 per 100,000 for CD in Italy [16]. Canada displays similar prevalence rates, with 248 per 100,000 for UC and 319 per 100,000 for CD [8]. Despite the emerging trend of increased IBD prevalence in Asian countries, these countries demonstrate much lower prevalence. For example, UC prevalence in Japan increased from 7.85 per 100,000 to 63.6 per 100,000 from 1984 – 2005. Similarly, CD prevalence in Singapore has increased

from 1.3 to 7.2 cases per 100,000 [10]. This increase is attributed to the westernization and industrialisation of these countries or an improvement in healthcare systems providing earlier diagnoses and a greater availability of databases to capture disease prevalence.

### 1.3.3 Public health relevance

Despite a plateau in incidence at the onset of the twenty-first century in the western world, recent data in high prevalence countries such as Canada, show increasing incidence driven by adult and paediatric cohorts [17] whilst peaks in the elderly have been reported in European patients [12].

Overall, regardless of divergent rates in incidence and prevalence according to geographical location, IBD is increasing on a global scale [18]. The extensive body of epidemiological data highlights the growing problem of IBD. The disease is incurable and significantly reduces patient quality of life, owing to the physical and psychological impact of the disease [19]. Available treatment options include 5-aminosalicylates, corticosteroids, immunomodulators (i.e. thiopurines and methotrexate), biological agents [20], [21] and surgery [22]. All these treatment modalities are initiated with the aim of inducing and maintaining remission. Whilst medical management of IBD has improved, increased prescription of medications, in particular biologic agents, has brought about significant increase in healthcare costs [23]. Previous data on the healthcare costs of IBD in a UK cohort revealed mean treatment costs over a 6-month period of £1256 for UC and £1652 for CD [24]. Disease severity and hospitalisation was positively correlated with the financial cost. Consistent with this, a recent report estimates that the 2.5 – 3 million IBD patients in Europe generate a 4.6-5.6 billion euros annual healthcare cost [25]. Collectively these data highlight the significant burden that IBD places on both the individual patient and the healthcare system.

### 1.4. Aetiology

The aetiology of IBD remains poorly defined. However, it is widely accepted that the pathology has a multifactorial aetiology and manifests as a result of a complex interaction between genetic, environmental, lifestyle and immunological factors [26].

#### 1.4.1 Immunogenetics

Substantial developments in genetic analysis methods have been a key contributor to an increased understanding of IBD pathogenesis. A meta-analysis performed on twin studies reported concordance rates of 30.3% for monozygotic vs 3.6% for dizygotic in CD and 15.4% vs 3.9% for UC, confirming a key genetic role in the disease [27]. Moreover, large scale genome wide association studies (GWAS) have thus far uncovered 163 genomic loci associated with the onset of IBD [28]. Consistently identified alleles evidence the role of autophagy in IBD pathogenesis. Dysregulation in this protein degradation mechanism impairs cellular homeostasis and is present in a range of pathophysiological conditions [29].

**NOD2**

The Nucleotide-binding oligomerization domain containing 2 (NOD2) is predominantly implicated in Crohn's disease susceptibility [30]. The NOD2 protein plays an integral role in the innate immune response [31]. NOD2 protein stimulation via peptidoglycans from microbiota leads to the activation of the autophagy pathway in a RIP2 protein-dependent manner via the mitogen activated protein kinase (MAPK) [32] and extracellular-signal-regulated kinase (ERK) [33] signalling cascades. The NOD2-RIP2 signalling cascade also activates NF-KB signalling, which mediates a Th17 immune response resulting in IL-22 activation and enhanced epithelial barrier function via production of antimicrobial peptides

(AMP), mucins and defensins [34]. NOD2 – NFKB signalling is inhibited in NOD2 mutants [35]. Consistently, CD patients exhibit reduced α-defensin concentrations [36].

**ATG16L1**

NOD2 can also stimulate autophagy independently of RIP2 via the interaction and recruitment of autophagy-related protein 16-like1 (ATG16L1) to the plasma membrane [37]. ATG16L1 plays an important role in autophagosome assembly and has been identified as a susceptibility variant for CD [38]. ATG16L1 encoding threonine at amino acid position 300 (ATG16L1*300T) confers protection against CD, whilst ATG16L1 encoding for alanine (ATG16L*300A) confers susceptibility to CD. HeLa cells expressing the ATG16L*300A demonstrate an impaired bacterial capture within autophagosomes following S.Typhinmurium infection compared to ATG16L1*300T. Additionally, in a human gut epithelial cell line, CaCo2 cells expressing ATG16L*300A failed to rescue autophagy of A.Typhimurium during ATG16L1 ablation compared with ATG16L*300T, which was able to mediate salmonella autophagy [39]. In line with this, ATG161 deficient mice exhibit heightened concentrations of proinflammatory cytokine IL-1β and acute colitis [40] highlighting the pathological effects of autophagy dysregulation.

**IL-23R**

The Interleukine -23 receptor (IL23R), a type I cytokine receptor, has been repeatedly implicated in IBD pathogenesis during GWAS [41].The binding of the IL23 protein to its receptor IL23R initiates a protective THimmune response mediated via JAK2 and STAT3 dependent mechanisms[17]. This results in production of IL-22 and also IL-17 [42], and subsequent neutrophil recruitment to the site of insult. Pidasheva and colleagues investigated the $IL23R^{Q381}$ and the $IL23R^{R381}$ variants in primary T cells from healthy volunteers. $IL23R^{Q381}$

showed reduced STAT3 phosphorylation upon stimulation with IL-23. In addition, $IL23R^{Q381}$ donors had reduced IL-17 and IL-22 concentrations. This data provides evidence that $IL23R^{Q381}$ is a loss of function allele and the defective immune response observed in this variant supports the role of IL23R in IBD pathogenesis [43]. To this extent, ustekinumab an antibody to the p40 subunit of IL12/23 has recently been licenced in the EU and North America for the treatment of moderate-to-sever Crohn's disease [44]. Risankizumab, an antibody targeting directly IL-23 signalling is presently under investigation [45].

Collectively, the mutations / single nucleotide polymorphisms (SNP) result in the dysregulation of the immune responses mediated via the gene products. Chronic impairment of these responses inhibits autophagic processes and impairs the secretion of protective proteins such as defensins and AMP's. This leads to epithelial barrier dysfunction, aberrant antigen exposure and a chronic inflammatory environment [46].

### 1.4.2 The Gut Microbiome

The gut houses the largest microbial community within the entire human organism, collectively referred to as the gut microbiome (GMB). The microbiomes genetic content exceeds cells of the human body by 100-fold [47] and plays a role in short chain fatty acid metabolism (SCFA) , prevention of pathogen invasion and preservation of the epithelial barrier [48]. The GMB is derived at birth during parturition and stabilises at around 10 years of age. It is largely composed of Firmicutes and Bacteroidetes, but demonstrates extreme inter-individual variation. Family members sharing the same environment show a highly variable microbiome composition [49] and it is currently unknown what constitutes a healthy microbiome [50]. The GMB is highly malleable and adapts to environmental stimuli. For example, the GMB composition of obese subjects adapts alongside $VO_2$ max and body composition following a period of endurance training [51]. Furthermore, significant reductions in bacteroides,

bifidobacterium spp, Escherichia coli and enterobaacteriasceae spp have been reported between subjects on a vegan diet and subjects on an unrestricted liberal diet [52]. This demonstrates that GMB composition is also influenced by dietary pattern.

### 1.4.3 Dysregulation of the gut microbiome in IBD

Early rodent models provided evidence for the role of the GMB in IBD. IL-10 knockout mice housed in germ free conditions display no evidence of colitis or immune system activation as opposed to IL10 knockout mice maintained in specific pathogen free (SPF) environments [53] thus, establishing a role for enteric bacteria in the development of colitis in this model**.**

In addition, many of the gene mutations identified via GWAS which confer susceptibly to IBD encode for proteins with functional roles in the recognition and processing of pathogens and stimulation of an immune response.

### 1.4.4 Characteristics of the microbiome in IBD

IBD patients exhibit an upregulation in pathogenic bacteria concomitant to a reduction in commensal bacteria, referred to as dysbiosis [47]. Microarray analyses of faecal samples from CD patients has evidenced reduced diversity of Eubacterium rectale relative to healthy controls [54]. Consistently, a metagenomic analyses of faecal samples revealed reduced firmicute diversity in CD to controls [55]. Available data from analyses of biopsy samples, which is the preferred method for analysing microbiota as appose to using stool samples, demonstrates an increased diversity of Firmicutes [56] which is in contrast to data from stool samples [55].

The magnitude of GMB perturbation is influenced by disease location. For instance, the GMB composition of the third and final part of the small intestine. (Ileum)in CD patients differs significantly from colonic CD patients and healthy controls [57]. Additionally, tissue

inflammation further influences GMB composition. Non-inflamed biopsy tissue taken from IBD patients exhibits an increased bacterial diversity relative to controls, whilst bacterial diversity is reduced in inflamed tissue [54].

It remains unknown whether these perturbations in microbial composition are caused by the inflammatory environment in IBD patients or whether the dysbiosis is a driver of the disease [34].

It is now established that the pathogenesis of IBD entails genetic susceptibility to the occurrence of aberrant microbiota sensing in the epithelium and the initiation and perpetuation of a defective immune response. For instance, IBD patients display mucosal T cell responses to commensal bacteria [58]. This aberrant pathogen sampling confers damage to the epithelial barrier, promoting continued antigen exposure in the host and loss of tolerance to commensal bacteria [59], further perpetuating this pathogenic cycle [60].

## 1.5 Factors associated with Inflammatory Bowel Disease

### 1.5.1 Psychological comorbidities

Available epidemiological data demonstrate a high prevalence of psychological illness in IBD. These comorbidities influence both the onset and clinical course of the pathology [61].Walker and colleagues examined the prevalence of anxiety and depression disorders in IBD. Major depression was significantly more common in IBD patients compared to controls (27.2% vs 12.3%), and panic disorders were also significantly more common in IBD patients (8% vs 4.7%) [62].

Moreover, abnormal self-reported baseline depression scores predated a diagnosis of CD rather than UC in 373 IBD cases (170 CD, 203 UC) from the Nurses' health study cohorts [63].

Psychological comorbidities also influence the clinical course of the disease. Mittermaier and colleagues reported a significant correlation between baseline anxiety & depression scores and disease relapse at 12 and 18 months follow up in 60 IBD patients with active disease [64]. In line with this, depression at baseline in a sample of 100 CD patients treated with infliximab was associated with a lower rate of remission and reduced time to re-treatment [65].

Collectively these data evidence the high prevalence of depression and anxiety in IBD and the potential cross-linking between disease activity states and psychological states in patients.

### 1.5.2 Sleep

Sleep disturbance is a chronic problem in IBD patients and there is a complex relationship between inflammation and sleep.

Ananthrhakrishnan *et al* investigated sleep disturbance in a cohort of 3,173 IBD patients consisting of 1798 patients in remission (507 UC and 1291 CD) and 1375 patients with active disease (587 UC and 788 CD) [66]. 60% of the entire IBD cohort had disturbed sleep at baseline. Multivariate analysis revealed that depressive symptoms and active disease were the strongest predictors of sleep disturbance. However, 50% of the 1291 CD patients in remission at baseline also exhibited sleep disturbance. Therefore, sleep difficulties are not restricted to patients with active disease.

Consistent with psychological illness, sleep disturbance may have an effect on disease course. In quiescent patients, 22% with disturbed sleep at baseline were found to have relapsed at 6 months follow up compared to 12% without sleep disturbance. Multivariate analysis revealed sleep disturbance was associated with a 2-fold increased risk of relapse at 6 months follow up in CD (OR, 2; 95% CI, 1.45 – 2.76). It may be difficult to explain any potential effect of sleep on disease outcomes. Certainly, symptoms predating a disease relapse may have an effect on

sleep pattern. Additionally, faecal incontinence, abdominal pain and concerns over potential stoma leakages will have an effect on sleep pattern and may be potentially commoner in patients with more active disease states.

Data from human models have revealed that high dose endotoxin administration increases wakefulness and suppresses non-rapid eye movement sleep (NREM) [67]. Whilst acute administration of IL-6 supresses rapid-eye movement sleep (REM) sleep but has no effect on NREM sleep and wakefulness [68]. These data provide evidence that the sleep cycle is influenced by host defence activation. Thus, in addition to the aforementioned symptoms and concerns, the aberrant immune response and pro inflammatory environment present in IBD, likely influences sleep disturbance.

### 1.5.3 Physical activity

Available epidemiological data may indicate reduced physical activity as having a possible aetiological role in the development of IBD. Sonnenberg *et al* [69] report reduced IBD prevalence among males performing job roles associated with higher physical activity, such as construction workers, when compared to more sedentary job roles such as locksmiths. A consistent trend was reported in females, where IBD incidence was higher in those working in office-based occupations compared to cleaners. In agreement, Khali *et al* later analysed physical activity data from the Nurses' health study cohorts [70]. The absolute risk values of developing IBD in females categorised in the highest fifth of physical activity was 8 and 6 per 100,000 compared to 11 and 16 per 100,000 women in the lowest fifth of physical activity, with a corresponding multivariate adjusted hazard ratio of 0.64 (95% CI 0.44-0.94 $P = 0.02$). These data highlight a possible role for reduced physical activity in the pathogenesis of IBD.

### 1.5.4 Malnutrition

Malnutrition is a severe complication in IBD and occurs in up to 85% of IBD patients independent of disease activity [71] . In hospitalised patients, malnutrition is more prevalent in CD, where up to 75% of patients are malnourished and 50% are in negative nitrogen balance [72].

The characteristics of malnutrition in IBD include micronutrient deficiencies and pathogenic alterations in body composition, stemming from protein-energy malnutrition [73]. Multiple factors contribute to malnutrition in IBD, including malabsorption resulting from gut resection, appetite reduction, protein loss and classic symptomology such as vomiting, diarrhoea and abdominal pain [74]

**Iron**

Iron deficiency is the main driver of anaemia in IBD cohorts and is present in 30-90% of patients presenting with anaemia [71]. Anaemia has been reported in 24% of CD and 18% of UC patients in a cohort of 200 Brazilian outpatients (100 CD and 100 UC). Both moderate disease activity (OR 3.49 , 95% CI 1.95 – 9.6) and elevations in the inflammatory marker serum C reactive protein (CRP) (OR 1.8, 95% CI 1.04 - 3.11) were associated with anaemia in these patients [75].

Higher prevalence rates have been reported at the time of IBD diagnoses. In a group of 1278 IBD patients, 41.2% of patients were anaemic at diagnosis (47% CD, 33.8% UC). Consistently, anaemia was associated with elevated CRP concentrations in CD (OR 4.08 95% CI 2.39-6.97) and UC (OR 4.58 95% CI 2.26-9.27) [76]. These increased prevalence rates likely reflect the treatment naïve status of these patients.

**Vitamin B12**

Vitamin B12 deficiency can inhibit nervous system function and is associated with a decline in cognitive function, anaemia [77] and fatigue [78]. In a sample of 257 IBD patients (195 CD, 62 UC), a higher prevalence of Vitamin B12 deficiency was found in IBD patients compared to controls (14.9% vs 4.2%) and also in CD compared to UC (14.9% vs 3.2%) [79]. This has been attributed to malabsorption driven by the inflammatory environment in the mucosal layer [80] and terminal resections with consequent B12 malabsorption in CD.

**Vitamin D**

Vitamin D deficiency is defined as 25-hydroxyvitamin D level <20 ng/ml [81] and plays an important role in a number of physiological processes including bone metabolism [82]. Vitamin D deficiency impairs muscle strength [83] and increases the risk of osteopenia and subsequent osteoporosis [84]. IBD patients exhibit a higher prevalence of Vitamin D deficiency relative to healthy unaffected people. Further, vitamin D concentrations are inversely related to disease severity [85]. Moreover, epithelial Vitamin D receptor signalling has been shown to regulate inflammation of the mucosa [86]. Consistent with this, Cyp and VDR knock out (KO), which inhibits Vitamin D synthesis, results in dysbiosis of the GMB [87] in a rodent model.

**Dietary factors**

Long term dietary fibre intake is inversely correlated to risk of CD incidence [88]. Early data from newly diagnosed CD patients characterised a pre-illness diet high in refined sugar, low in fibre and reduced fruit and vegetable consumption relative to controls [89]. Pre-treatment of human adenocarcinoma cells with the SCFA butyrate, inhibits Tumor necrosis factor antagonists (TNF-α) induced phosphorylation and degradation of IKB, subsequently blocking

NF-Kb translocation [90]. In keeping with this, administration of butyrate enemas in six UC patients was shown to inhibit NF-kappaB translocation in lamina propria macrophages and was associated with a reduction in disease activity [91]. Additionally, non-starch polysaccharide (NSP) preparations from plant-based dietary fibre, such as Plantain, inhibits E-Coli translocation across M cells & Peyers patches [92] which is consistent with the inverse relationship between fibre intake, CD incidence [88] and the reduced fibre consumption shown in newly diagnosed patients [89]. In addition, the commonly used food emulsifier polysorbate-80, increases E-Coli translocation through M cells & Peyers patches [92]. Which perhaps provides a mechanism behind the association between IBD incidence and the western diet [93].

### 1.5.5 Symptoms & Characteristics

Clinical manifestation of IBD is dependent upon disease location and severity. UC is restricted to the colon and patients can be subcategorised into procitis, left sided colitis or pancolitis [94].In contrast, CD is transmural and presents in any area of the GIT from mouth to anus. Distribution of CD varies, 25% of patients present with disease in the colon, 25% ileitis and 50% of patients have ileocolitis. A further 33% present with perianal CD and 5-15% present with oral or gastroduodenal disease [95].

CD is also characterised by formation of fistulas, abscesses and strictures of affected areas within the GIT. IBD patients typically present with a myriad of unpleasant symptoms including abdominal pain & cramping, increased urgency and stool frequency, bloody diarrhoea and passage of mucus [94].

## 1.6 Diagnosis

Diagnosis of IBD requires assessment of the patient using a range of serological, endoscopic and radiological methods.

### 1.6.1 Serological assessment

Initial blood tests includes full blood count, erythrocyte sedimentation rate, C-reactive protein, liver function as well as assessment of iron, vitamin B12 and Vitamin D levels [95]. Stool samples may be analysed for the faecal calprotectin and lactoferrin, which are biomarkers of inflammation within the GIT.

### 1.6.2 Endoscopy

Endoscopy is commonly used to confirm diagnoses of IBD and is the current gold standard measure of disease activity [96]. Both upper and lower GI Endoscopy are used to assess disease severity & location and provide the clinician with the opportunity to obtain a biopsy for further laboratory analyses.

### 1.6.3 Radiological assessment

Radiological imaging methods are now routinely used for both initial diagnoses of IBD and the monitoring of response to treatment. Whilst CT scanning is commonly utilised, a significant limitation is the exposure of the patient to ionising radiation. MR imaging overcomes this limitation. Both MR and CT methods demonstrate high sensitivities of 90.5% vs 95.2% in the detection of small-bowel CD when assessed against endoscopy [97].

## 1.7. Disease classification

### 1.7.1 Crohn's disease

The original classification of Crohn's disease was formulated by an international working party in Rome in 1991 [98]. This was based on anatomical distribution, operative history and clinical behaviour in terms of inflammation, fistulising and stenotic disease. Following this, the Vienna classification (VC) of CD was formulated by the world congress of Gastroenterology in 1998. This compromised age of onset, disease location and disease behaviour [99]. This was later revised in 2005, where the Montreal classification (MC) was proposed by the working party at the world congress of Gastroenterology [98] in an attempt to address a number of issues identified with the VC. The MC modification enables early onset IBD to be categorised separately, which was an important modification for disease classification in Paediatric cohorts. The MC also addressed the issue of the VC categorising location at 4 exclusive sites. Upper gastrointestinal disease can occur simultaneously with ileal and colonic disease. The * in MC allows L4 to be added to L1-L3 when presenting simultaneously with upper gastrointestinal disease (Table 1). The MC also addressed the issue of perianal disease not having a separate sub classification in the VC.

### 1.7.2 Ulcerative Colitis

Both the Rome & Vienna working parties failed to establish a separate classification system for Ulcerative Colitis. This was addressed at the MC, where a classification system based on the extent and severity of UC was formulated (Table 2 & 3).

The MC remains widely used and demonstrates excellent inter-observer agreement for diagnosis, perianal CD, CD location, age of onset, upper GI disease, CD behaviour, UC extent [100].

| | Vienna Classification | Montreal Classification |
|---|---|---|
| **Age at diagnosis** | A1 <40 years<br>A2 >40 years | A1 <16 years<br>A2 17-40 years<br>A3 >40years |
| **Location** | L1 Ileal<br>L2 Colonic<br>L3 Ileocolonic<br>L4 Upper | L1 Ileal<br>L2 Colonic<br>L3 Ileocolonic<br>L4 Isolated upper disease |
| **Behaviour** | B1 non-stricturing, non-penetrating<br>B2 Stricturing<br>B3 penetrating | B1 Non-stricturing, non-penetrating<br>B2 stricturing<br>B3 penetrating<br>P Perinal disease modifier |

***Table 1-1.*** *Comparison of the Vienna and Montreal classifications of Crohn's disease taken from Satsangi et al* [*98*].

**Montreal classification of extent of ulcerative colitis (UC)**

| **Extent** | | **Anatomy** |
|---|---|---|
| **E1** | Ulcerative proctitis | Involvement limited to the rectum (that is, proximal extent of inflammation is distal to the rectosigmoid junction) |
| **E2** | Left sided UC (Distal UC) | Involvement limited to a proportion of the colorectum distal to the splenic flexure |
| **E3** | Extensive UC (Pancolitis) | Involvement extends proximal to the splenic flexure |

***Table 1-2.****The Montreal classification of Ulcerative Colitis, taken from Satsangi et al [98].*

**Montreal classification of severity of ulcerative colitis (UC)**

| Severity | | Definition |
|---|---|---|
| **S0** | Clinical remission | Asymptomatic |
| **S1** | Mild UC | Passage of four or fewer stools/day (with or without blood), absence of any systemic illness, and normal inflammatory markers (ESR) |
| **S2** | Moderate UC | Passage of more than four stools per day but with minimal signs of systemic toxicity |
| **S3** | Severe UC | Passage of at least six bloody stools daily, pulse rate of at least 90 beats per minute, temperature of at least 37.5°C, haemoglobin of less than 10.5g/100ml, and ESR of at least 30mm/h |

***Table 1-3.*** *The Montreal classification of Ulcerative Colitis severity, taken from Satsangi et al [98].*

### 1.8 Disease management

A number of medications are used in the treatment of IBD with the aim of inducing and maintaining remission via mucosal healing. Remission can be subdivided into histologic, endoscopic and symptomatic categories. IBD patients usually begin with induction therapy and

once remission is reached, maintenance therapy is used in an attempt to negate relapse for as long as possible [95].

5-Aminosalicyclic acid (5ASA) medications, including Mesalamine & Sulfasalazine are available to treat IBD. The mechanism behind 5-ASA's therapeutic effect remains unknown. However, they are known to inhibit arachidonic acid metabolism and thus downregulate prostaglandin and leukotrienes [101].

Thiopurine therapy is commonly used in the treatment of IBD for their immunosuppressive capabilities. Azathioprine is metabolised to 6-mercaptopurine (6-MP) within erythrocytes. Hypoxanthine phosphoribosyl transferase (HPRT) enzyme then converts 6-MP into 6-thioguianine nucleotide (6-TG) [102]. This purine antagonist impairs DNA and RNA synthesis and inhibits T and B lymphocyte synthesis [103].

Methotrexate is a folate antagonist widely used to treat IBD which has proved effective in inducing [104] and maintaining remission [105] when administered at low dosages in CD cohorts. Methotrexate's potent anti-inflammatory effect results from increases in intracellular AIRCAR concentrations, which promotes extracellular adenosine release. This exerts a suppressive effect on inflammation via recruitment of $A_2$ receptors to the site of inflammation and subsequent downregulation of leukocytes [106].

More recently, TNF-α antagonists have proved highly effective in the treatment of IBD. Available TNF-α inhibitors include infliximab and adalimumab. These chimeric monoclonal antibodies against TNF-α are able to bind and neutralise TNF-α [107]. Infliximab has been demonstrated to induce apoptosis in lamina propria T cells [108] and inhibit cytokine secretion of IL-6 and IL-8 [109]. The efficacy of anti-TNFAα inhibitors is well-established during combined immunosuppression with corticosteroids [110] .

Anti-adhesion molecules also show promise in the treatment of IBD. Vedolizumab, a humanised immunoglobulin G1 monoclonal antibody to the $a_4\beta7$ integrin, has shown efficacy in both the induction and maintenance of remission in CD patients [111]. Vedolizumab downregulates gastrointestinal inflammation via inhibiting the interaction of $a_4\beta7$ integrin and mucosal addressing cell adhesion molecule-1 (MAdCAM-1) [112].

Consistently, Ustekinemab is a monoclonal antibody to the p40 subunit of IL12/23 which has proved effective in inducing and maintaining remission in moderate to severe CD [44]. Ustekinemab prevents IL-12/23 from binding to the IL-12RB1 receptor chain of IL-12 (IL-12RB1/B2) and IL23 (IL-12RB1/23R) receptors located on T cells which inhibits IL-12/23 signalling and subsequent cytokine secretion [113].

Furthermore, restoration of dysbiosis in the GMB via Faecal microbiota transplantation (FMT), which involves replacing the dysregulated microbiome of IBD patients with a healthy normal microbiota from a healthy donor [50] demonstrates potential efficacy in the treatment of IBD. A recent multi-centre randomised, double-blind placebo-controlled trial of FMT in active UC patients reported steroid free endoscopic remission in 55% FMT group vs 17% autologous FMT [114].

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# Chapter 2 – The aetiology of fatigue in Inflammatory Bowel Disease (IBD)

### 2.0 Introduction

The purpose of this chapter is to provide a general overview of fatigue, prior to a review of available data specific to IBD fatigue. The first part of the chapter will introduce the general constructs of fatigue, including relevant definitions, methods for quantifying this multifactorial symptom and contemporary issues within the existing literature base. This will provide a foundation from which the aetiology of the fatigue that manifests in IBD can be understood. The next section will highlight the paucity of data available on aetiological factors in IBD fatigue and provide an overview of relevant physiological data from the study of IBD patients. In the absence of objective data on IBD fatigue aetiology, these data can aid in identifying potential mechanisms that modulate this pervasive symptom. In turn, highlighting potential targets for mechanistic investigations. Finally, currently available management strategies to target IBD fatigue are reviewed. In doing so, this will highlight the fundamental rationale for the experimental work presented in the later chapters of this thesis.

*The work within this chapter has been derived from the following review article published in July 2021 [1]*

### 2.1. Fatigue Overview

Fatigue has been recognised as a problematic physiological symptom since the seminal work of Angelo Mosso in the late 19$^{th}$ century [2]. Despite a plethora of research pertaining to fatigue spanning the last 130 years, the aetiology is poorly understood and there remains no consensus

definition of fatigue.

Fatigue is a complex, multi-dimensional symptom mediated via the integration of the central nervous system (CNS), cardiorespiratory and musculoskeletal systems. Exercise fatigue is defined as the failure to achieve or maintain an expected work output [3]. Whereas perceived fatigue describes the subjective perception of tiredness, often disproportionate to physical or cognitive demand, which is common in chronic disease. The difficulty in delineating fatigue aetiology likely relates to the broad manifestation and general complexity of the symptom. Although the fundamental absence of a consensus definition undoubtedly exacerbates the issue. As a result, data on fatigue spans many scientific sub disciplines, ranging from the investigation of exercise-induced decrement in skeletal muscle function [4] through to qualitative examination of subjective sensations such as tiredness and exhaustion in patient cohorts [5]. These two scenarios clearly represent attempts to delineate distinct physiological processes.

This compartmentalisation of fatigue research [6] across exercise physiology [7], medicine [8] and psychology [9] may be considered necessary to fully elucidate fatigue aetiology. However, this segregation has thus far failed to delineate fatigue aetiology. Furthermore, the lack of consensus definition means that the aforementioned disciplines ascribe varying working definitions to fatigue, leading to inconsistency within the literature. A notable example highlighted by Kluger *et al* is that of "Central fatigue" which in the context of psychology research can refer to decrements in cognitive task performance and motivation [10]. Concomitantly, the same terminology is ascribed to physiological perturbations at the level of brain and spinal cord, which impede skeletal muscle contractility and cause exercise fatigue [11]. Recent taxonomies of fatigue have highlighted these inconsistencies and recommended to omit discipline specific terminology and standardise experimental models across laboratories [6] to facilitate a greater understanding of the symptom. Successfully

characterising fatigue aetiology across health and disease therefore requires a clear definition of the symptom combined with appropriate functional and mechanistic assessments.

## 2.2 Fatigue Taxonomy

Fatigue is classically attributed to “central” and “peripheral” origins originally proposed by Mosso [2]. Whereby physiological perturbations occurring at the level of the brain & spinal cord (central fatigue), or at the neuromuscular junction and the skeletal muscle itself (peripheral fatigue) result in acute, transient performance decrement. This classical view of fatigue aetiology predominates across the available literature, where experiments around fatigue mechanisms are approached solely from the central and peripheral level. Concomitantly, psychological based investigations of fatigue attribute “central fatigue” to a reduction in subjectively measured motivation or quantitative measurements of cognitive function.

In reality, whether manifesting as an acute exercise-induced impairment in skeletal muscle function, cognitive performance deficits or a chronic perception of exhaustion, these collective symptoms are fundamentally intertwined. They likely represent a complex interlinking of multiple physiological systems. Whilst it has recently been suggested to omit the original central and peripheral dogma [6], outlining the mechanisms underlying these central and peripheral alterations is helpful to facilitate an understanding of fatigue.

### 2.2.1 Fatigue Perception

There are multiple domains of fatigue perception [12] linked to differential mechanisms. IBD patients report increased fatigue perception in the physical and cognitive domain [13] (i.e., the perception of reduced performance in physical and cognitive tasks), which is modulated by the contralateral sensorimotor cortex [14] and the frontoparietal network [15]. Similarly,

motivational fatigue occurs in IBD and has been associated with altered orbitofrontal cortex activity, due to its role in decision making [16]. The perception of fatigue during both resting conditions and exercise tasks is known to result from alterations in core temperature [17]and hydration status, together with metabolite and neurotransmitter metabolism [18-22]. Similarly, it is now recognised that psychological factors such as awareness [23] [24] [25] arousal level, external input [26] and motivation strongly contribute to the fatigue perception.

### 2.2.2 Measuring fatigue severity

Fatigue symptoms are typically assessed retrospectively in a chronic context relating to the individual's symptomology over a preceding period. This is done by self-reported scales, which assess fatigue as a trait level variable. Similarly, fatigue can be assessed during or following task performance, as a state level variable [27]. These self-reported scales are the only way to quantify the severity of fatigue symptoms across domains [5]. Objective methods used to assess exercise fatigue (e.g. loss of muscle force as a function of time) do not inform on the severity of subjective fatigue perception experienced by an individual.

The majority of self-report fatigue scales assess fatigue as a trait level variable. Where the individual responds to standardised questions relating to their perception of fatigue during a period preceding the exam [6]. The checklist individual strength scale (CIS) was developed by Vercoulen and colleagues and is a prominently used to assess trait level fatigue. The scale consists of four sections assessing subjective fatigue and reductions in motivation activity and concentration. The assessment of these domains compromises twenty statements rated on a seven-point scale pertaining to their experience of fatigue over the preceding 14 days, with higher scores indicating a greater severity of fatigue within the specified domain [28]. The CIS has been shown to reliably quantify fatigue characteristics across cohorts of patients in a study comparing MS, CFS and healthy volunteers [29]. Similarly, another prominent instrument for

fatigue assessment including is the multidimensional fatigue inventory 20 (MFI-20) [12] which has been applied in IBD cohorts [30]. The scale assesses fatigue across five dimensions including general fatigue, physical fatigue, mental fatigue, reduced motivation and reduction activity. Across a series of 20 items. Each item is rated on a 5-point scale and consists of a statement such as "I feel fit", with a graded scoring system ranging from 1 "yes that is true" through to 5 "no, that is not true". If the subject agrees entirely with the statement, they are instructed to mark box number 1 on the left of the statement. If a volunteer disagrees with the given statement, they are instructed to mark the boxes in the direction of the opposing statement. Total scores from items within each of the 5 domains are summed to give a score between 4 and 20, with a higher score reflecting higher perceived fatigue within that domain in the period preceding completion. The validity of the scale has been demonstrated in numerous cohorts including cancer patients [31] CFS patients, healthy volunteers [32] and more recently in liver transplant [33], ICU [34] and IBD patients [30]. The sensitivity of the General fatigue dimension has previously been highlighted during the assessment of psychometric properties of the MFI-20 scale in healthy controls [12] and patient cohorts [31]. The sole use of the General dimension is recommended in scenarios where a brief fatigue measurement instrument is required [12, 31] (e.g. a clinical trial) and previous experiments in IBD have reported data solely from the general [35] and the physical domains [13] of the MFI-20. This approach is replicated in the data within the later experimental chapters of this thesis.

Despite the relative simplicity of self-reported fatigue assessment, determining clinically significant fatigue perception is problematic [10, 36] . Generally, a cohort are classified as "fatigued" when their self-reported scores are significantly elevated relative to a healthy control group [37].Previous IBD experiments have defined clinically significant fatigue by calculating a threshold based on the 95$^{th}$ percentile of the fatigue scores in the general population [35, 38, 39] or an internal healthy control group [39] whilst others have used the 75$^{th}$ percentile of

general population [40] scores [13].The stratification of IBD patients into fatigued and non-fatigued subgroups using self-reported scales has revealed that patients with heightened fatigue complaints are deconditioned (i.e. reduced cardiorespiratory fitness), weaker (i.e. less peripheral muscle strength [41]) have an altered immune profile [42] and differential faecal microbiota composition [43] relative to non-fatigued patients. These data are useful as they highlight potential aetiological factors in IBD fatigue. However, a variety of assessment scales are used to assess fatigue perception [44]. Whilst there is some consistency between studies [42], [41] many groups use different scales [43]. This is likely due to the lack of consensus on fatigue measurement, even within the same disease. This impairs harmonisation of data sets across multiple cohorts. Another contemporary issue is the use of fatigue assessment scales that have not been validated for use in the specific cohort. Whilst there are common correlates of fatigue in chronic disease such as physical deconditioning (i.e. a reduction in $VO_2$ peak and or peripheral muscle function), the magnitude varies across conditions [45, 46]. Pharmacological treatments also influence fatigue state to varying magnitudes across chronic disease. For example, chemotherapy induces severe fatigue in cancer patients [47], as does thiopurine therapy in IBD patients [48], although this transiently resolves following cessation of thiopurine use, whereas chemotherapy related fatigue does not. Moreover, secondary disease symptomology and comorbidities can also co-occur with fatigue perception or mimic symptomology. This make it difficult to separate the symptoms, this is a particular problem for depression and anxiety [49]. Given these factors, and the distinct pathophysiology of chronic conditions, there is a requirement for the development and validation of disease specific instruments in order to accurately and reliably quantify the magnitude of fatigue perception in a given cohort. The inflammatory bowel disease fatigue questionnaire (IBDF-scale) was developed in response to the high prevalence of fatigue seen in IBD cohorts. The scale has been found to accurately assess fatigue in a cohort of 465 IBD patients, when compared against the

MFI-20 and multidimensional assessment fatigue scale [50]. However, McNelly *et al* compared the effect of increased physical activity and / or omega-3 fatty acid supplementation on fatigue in a cohort of 52 quiescent IBD patients [51]. Following the 12-week intervention, fatigue assessed as a primary outcome using the FACIT-f worsened with omega-3 supplementation ($P$=0.02) with no effect of exercise advice compared to placebo ($P$=0.38). Conversely, when fatigue was quantified using the IBD-F scale as a secondary outcome, exercise advice significantly decreased fatigue compared to placebo ($P$ = 0.03) with no effect observed for omega-3 supplementation ($P$=0.42) [51]. These divergent data between different fatigue scales, both of which are validated to assess trait level fatigue specifically in IBD patients (that is fatigue burden over a preceding 7-day [52]or 14-day [53] period prior to completion), bring into question the convergent validity of these instruments and highlight the pitfalls of subjective fatigue assessment. 252 self-report fatigue assessment scales have been identified, 150 of which have been only been used in a single experiment [44].

Collectively these findings demonstrate the need for a consensus approach on standardised measurement of perceived fatigue burden in IBD using validated scales, alongside the measurement of exercise fatigue during relevant markers of physical function.

### 2.2.3 Fatigue perception in IBD

In addition to the premature exercise fatigue present in IBD, a heightened perception of fatigue across multiple domains is a common secondary disease symptom. Affected patients have reported the perception of heavy limbs [13] and disrupted cognitive function [13] indicating the presence of fatigue in the physical, cognitive and motivational domains. In the general population, the symptom of fatigue is associated with increased mortality [54] and impairs health-related quality of life in IBD [55]. The impact of this pervasive secondary symptom is illustrated by the data showing that IBD patients perceive fatigue burden to be greater than that

of cardinal disease symptoms such as bowel urgency and diarrhoea [56]. Despite this, fatigue is not prioritised in clinical consultations [57] and the symptom remains poorly understood by both clinicians [58] and patients [59].

Fatigue is associated with absenteeism [60] [61] and is the commonest reason for work absence in IBD (51%), exceeding that of medical appointments (49%) and abdominal pain (46%) [62]. Consistently, unemployment is higher in IBD patients than healthy controls and patients also retire earlier [61]. Limited employment status is also associated with IBD fatigue (2.50, 1.16-5.39, $P$ = 0.02) [63]. This is an important consideration as low socio-economic status is associated with increased hospitalisation and mortality in Crohn's disease [64]. Clearly, there is a negative socioeconomic impact of IBD fatigue.

The prevalence of increased fatigue perception in IBD has been reported as high as 86% in active disease [35] whereas 50-52% of patients with inactive or mild disease report substantial fatigue relative to 22% of healthy controls [37].The persistence of fatigue symptoms into disease remission is consistent with other autoimmune disease [65]. The recent prioritisation of IBD fatigue research by the Nurses-European Crohn's and Colitis Organisation (N-ECCO) and the IBD priority setting partnership with the James Lind alliance, [66] is a positive development, however there remains no conclusive data on treatment options available for patients [67] [35, 68] [69] as the underlying aetiology is unknown.

### 2.3 Exercise fatigue

Sustained exercise is maintained via integration of central nervous system (CNS), cardiorespiratory and musculoskeletal systems [70]. Neural drive to skeletal muscle regulates muscle fibre activation and perturbations within the neuromuscular pathway can lead to force loss termed supraspinal or central fatigue [71]. This can result from reduced neural output from the motor cortex and spinal motor neurons, as well as reduced neuromuscular junction

transmission. The centrally mediated mechanisms originating at the level of the brain or spinal cord [71] were initially characterised when suboptimal cortical output was evidenced across a range of contractile intensities using electrophysiological techniques [71]. These data established that voluntary output elicited during skeletal muscle contractions was not maximal. This involuntary reserve is mediated via central inhibitory mechanisms, which function to limit force output.

Simultaneously, peripheral fatigue originating at the intramyocellular level can occur from predominant anaerobic ATP synthesis and metabolite accumulation associated with high contractile intensities, or as a result of substrate depletion [72, 73] during prolonged exercise. The resultant inhibition of contractile mechanics and loss of muscle force is termed "bioenergetic failure". In chronic disease, these physiological fatigue processes at the central and peripheral level are often potentiated relative to healthy control cohorts [74].

### 2.3.1 Measuring exercise fatigue

Exercise fatigue can be quantified during laboratory exercise, which due to methodological restrictions, generally involves either isometric contractions across a spectrum of relative intensities or repeated maximum isokinetic contractions. The application of electrophysiological measurements during such tasks [75, 76] or during pre-post exercise measurements following dynamic exercise modalities [17, 77, 78] can delineate contributions of myoelectrical failure to force loss [71].

During sustained maximum voluntary contractions (MVC), the majority of force loss (exercise fatigue) is due to mechanisms directly at the skeletal muscle level, whilst reductions in central drive assessed via transcranial magnetic stimulation (TMS) accounts for only 25% of force loss [71]. In contrast, during prolonged submaximal contractions, centrally mediated brain fatigue arising from reduced motor cortical output accounts for 50-66% of exercise fatigue [79]. This

is consistent with fatigue processes measured during whole-body dynamic exercise. During cycling time trials, peripheral fatigue assessed via the reduction in potentiated twitch force from motor nerve stimulation was 40% following a 4km time trial, relative to 31% in a 20km trial and 29% in a 40km trial. Further, voluntary activation, quantified via cortical stimulation, reflecting centrally mediated fatigue, was -6% for the 4km time trial, compared to -12% for 20km and -10% for 40km trial. Thus, greater peripheral fatigue occurs during shorter duration, higher intensity exercise whilst alterations in the CNS cause exercise fatigue during sustained submaximal exercise [80].

Whilst the predominant mechanisms contributing to force loss depend upon task specificity, it is important to consider that central and peripheral alterations co-occur. For example, both the motor evoked potential ($P < 0.05$) and potentiated twitch torque ($P < 0.05$) were reduced during TMS and motor nerve stimulation ($P < 0.05$) following marathon running in trained volunteers [81]. The interaction between these alterations regulates exercise fatigue. For example, maintaining post-exercise metabolite concentrations in the elbow flexors by occluding limb blood flow, maintains group III/IV muscle afferent signalling into the CNS. This continues to supress motoneuronal output and inhibit voluntary activation, until after blood flow is reinstated and metabolites are cleared [71]. Consistently, the attenuation of muscle afferent feedback from skeletal muscle to the CNS alters exercise performance. Intrathecal fentanyl administration increases central motor drive (assessed by quadriceps iEMG) ($P < 0.05$) and muscle power output ($P < 0.05$) relative to placebo, during the initial 2.5km of a 5km cycling time trial. However, during the final 2.5km, central motor drive in the fentanyl group became consistent with placebo ($P =0.39$) whilst muscle power reduced ($P<0.05$) and both blood lactate ($P <0.05$) and quadriceps fatigue increased ($P < 0.001$) [82]. These data demonstrate the potentiation of exercise fatigue via inhibition of muscle afferent signalling to the CNS.

Generally, the limited usefulness of laboratory-based fatigue assessment underlies the lack of progress made in delineating fatigue aetiology. Current electrophysiological methods are only able to quantify the primary loci of force loss in real-time during laboratory-based tasks or following completion of traditional exercise tasks. Similarly, methodological limitations of functional neuroimaging methods used to assess fatigue also complicate data interpretation. For example, BOLD is the measure of the haemodynamic response function within the brain [83] and can be used during scanner-based motor tasks to estimate neural activation. However, the technique cannot discriminate between excitatory or inhibitory neuronal activity. A primary issue in fatigue research is that it remains unknown how these laboratory data relate to real world performance [6] which is of particular relevance in chronic disease.

### 2.3.2 Peripheral muscle strength in IBD

Muscle strength and function is highly correlated with muscle mass [84]. Exercise fatigue during maximum strength-based tasks (e.g. muscle force loss during a sustained maximum contraction) is known to result predominantly from mechanisms at the skeletal muscle. Peripheral muscle strength is known to be reduced in IBD patients [85]. Maximal isometric knee extensor strength is less in IBD patients relative to healthy controls when assessed bilaterally and normalised to fat free mass. ($P < 0.001$) [86]and when assessed unilaterally and normalised to body mass ($P = 0.039$) [87]. Peak knee flexor isometric torque is also less in IBD relative to controls during  normalised to body mass ($P = 0.022$) [87]. This strength loss was linked to reduced neuromuscular function as a 51% reduction in peroneal nerve signal amplitude was reported in the Crohn's disease relative to healthy controls ($P = 0.067$).

Other studies have demonstrated that maximal isometric ($P < 0.001$) [86]  knee extensor endurance is less in IBD patients compared to healthy control volunteers. Interestingly, the greater rate of knee extensor fatigue during maximal repeated isometric contractions was

correlated with the self-reported fatigue perception in IBD patients assessed by the physical domain of the multidimensional fatigue impact scale (FIS) ($r2 = -0.52$, $P < 0.01$) [88]. Bilateral isometric knee extensor endurance has also been shown to be less in CD patients compared to healthy controls [86] . However, the rationale for the use of high-intensity isometric contraction to quanitfy muscle fatigueability is unclear. The venous occlusion associated with high intensity isometric contraction will confound the assessment of muscle fatigueability.

Whilst these measurements provide information on the maximum instantaenous strength capabilities of a large muscle mass, this is preferentially modulated by central drive [71] and skeletal muscle mass [84] thus providing little information on muscle fatigue as defined by an accelerated loss of maximal force output.

Regarding upper limb function, the handgrip strength of quiescent Crohn's disease patients remains comparable to healthy control cohorts when normalised to body cell mass and fat-free mass. However, handgrip strength was less in in a cohort of 50 UC patients in both absolute terms and when normalised to body cell mass ($P = 0.001$). [89] This loss of handgrip strength specific to UC patients could be related to the reduction in body cell mass in malnourished UC patients relative to well-nourished UC patients ($P = 0.044$) [89]. This finding was specific to UC and not observed in Crohn's disease patients. [89] In paediatric IBD, handgrip *z* scores remained consistent in a cohort with mixed disease activity [90] relative to healthy children. Although handgrip strength was reduced in a cohort of paediatric patients with quiescent to mild disease activity ($-0.34$ vs $0.83$, $P \leq 0.015$) [91].

Gender differences in exercise fatigue are well known [92]. Female Crohn’s disease patients demonstrated lower maximal isometric knee extensor strength and endurance when both were assessed bilaterally ($P < 0.05$) relative to male Crohn’s disease patients [86]. Consistently, both handgrip strength ($P < 0.05$) and endurance ( $P < 0.01$) were also reduced relative to male Crohn’s disease [86]. These data were normalised to FFM and this gender effect on exercise

fatigue was not observed in a healthy control cohort [86]. This is implicit of a gender effect in IBD exercise fatigue [85, 93]. Although as previously highlighted, the chosen measurements only reflect strength capabilities and do not reflect muscle endurance. Interestingly, female gender is a risk factor for greater fatigue perception in IBD [94]. Future work should aim to delineate the role of sex in IBD exercise fatigue and the relationship with heightened fatigue perception.

### 2.3.3 Whole-body deconditioning in IBD

Whole-body deconditioning (i.e. impaired cardiorespiratory fitness) is well established in IBD patients across a range of exercise modes. Incremental exercise testing using a bed adapted cycle ergometer revealed a progressive reduction in the peak aerobic workload attained in relation to the magnitude of proctocolectomy and resection [95]. The peak aerobic workload in patients without small bowel resection was reduced by 9% relative to reference values from healthy subjects. Peak aerobic workload was reduced by 22% in patients with moderate small bowel resection (15%-30%, $P < 0.01$) and by 40% in patients with >50% small bowel resection ($P < 0.01$). Similarly, pre-operative incremental exercise testing in IBD demonstrated that the blood lactate threshold occurs at a lower $VO_2$ in IBD patients relative to gender-specific reference values ($P < 0.0001$) [96]. The heart rate recovery of IBD patients following an exercise stress test was also shown to be longer than a healthy control group ($P < 0.001$) [97]. These collective findings evidence whole-body deconditioning in IBD, which appears to be linked to disease activity, given the magnitude of deconditioning increases with the extent of small bowel resection [95].

Congruent with these laboratory-based assessments of cardiorespiratory fitness, physical performance during sustained submaximal activities is also less in IBD. CD patients were found to be 25% slower than healthy controls during a repeated sit up test ($P < 0.001$) [86]

whilst UC patients were 32% slower ($P < 0.001$) and displayed a 17% reduction in gait speed ($P = 0.002$) relative to HV [93]. Consistent with this deconditioning, physical inactivity is reported across the lifespan in IBD. The accelerometer measured step count (11,166 [1060; 12,272] vs 9,828 [8937; 10,718]) and duration of physical activity (2.74 [2.33; 3.14] vs 2.29 [1.96; 2.63]) is lower in paediatric IBD patients relative to healthy age-matched controls [91]. Likewise, adult IBD patients performed less accelerometer counts ($1.3*10^6$ vs $2*10^6$) and less bouts of high-intensity exercise (1 vs 5) relative to HV ($P < 0.01$) [98]. Interestingly, despite comparable accelerometer measured physical activity between a cohort of CD and HV ($P = 0.45$) [99], when CD patients were stratified into a low and high phosphorylated: total Akt ratio group, reflecting impaired and normal muscle hypertrophy signalling, the low ratio group were less active compared to the high ratio group ($P = 0.009$). This reduction in physical activity is consistent with the 14% reduction in quadriceps cross sectional area reported in the CD group ($P = 0.055$) [99] and may represent inactivity induced muscle decline [100].

### 2.4 The aetiology of IBD fatigue

Despite the absence of quantitative data on IBD fatigue aetiology, there is a plethora of available data pertaining to IBD pathophysiology, which can help to highlight potential factors mediating premature fatigue development. The next section of this chapter will outline the pathophysiological factors in central and peripheral physiology that associate with IBD fatigue and in doing so, provide rationale for the work undertaken in this thesis.

#### 2.4.1 Sleep disturbance

Sleep difficulties [101] and reduced sleep quality [63] are associated with an increased risk of fatigue development across various domains of fatigue perception in IBD [63]. Available data show that 82% of Crohn's disease patients and 72% of UC patients report reduced sleep quality

during active disease. The persistence of sleep difficulties continues into disease remission as 51% of Crohn's disease and 47% UC patients with quiescent disease report difficulty sleeping [102]. Sleep difficulties in IBD likely relate to, at least in part, disease symptomology. Symptoms such as abdominal pain and urgency, faecal incontinence, all of which are likely to be worsened in patients with active disease will impede sleep pattern and quality. This will directly contribute to fatigue related symptoms such as daytime sleepiness [103].

From an in-direct perspective, there may be a bidirectional relationship between sleep difficulties, inflammation and disease activity, although limitations in the experimental design of available data limit resolute conclusions on this. Proinflammatory cytokines are linked to sleep disturbance. Interleukin-6 (IL-6) administration reduces sleep quality and evokes fatigue symptoms in healthy volunteers [104]. Likewise, sleep restriction results in elevated plasma IL-6 [105] and tumour necrosis factor alpha (TNF) concentrations [106]. It has been hypothesised that active IBD and the associated inflammatory burden may cause sleep difficulties and potentiate fatigue burden [107]. Indeed, quiescent patients with sleep difficulties have a greater rate of 6-month relapse relative to IBD patients without sleep difficulties [108]. Although it is difficult to demonstrate a causal link between these disease related factors and fatigue development. Moreover, other mechanisms must predominate in quiescent patients who present with sleep difficulties [102].

### 2.4.2 Inflammation

Inflammation is a known contributor to active disease fatigue [109]. Indeed, fatigue prevalence [110] and severity [111] are greater in active IBD relative to quiescent disease.

Fundamental to IBD, vagal sensory neurons are known to mediate gut-brain signalling and relay inflammatory signalling into the CNS [112]. At the peripheral level, pro-inflammatory cytokines such as TNF directly inhibit skeletal muscle contractile function [113, 114]. It is

known that this mechanism is mediated via the TNFR1 [115] as genetic TNFR1 deficiency prevents TNF induced increase in cytosolic oxidant activity and myofibrillar force loss observed in TNFR2 deficient mice and controls [115]. TNF infusion also evokes skeletal muscle insulin resistance by increasing phosphorylation of P70S6K, ERK1/2 NK and the IRS-1 on serine 312 ($P < 0.05$), and inhibiting AKTS160 phosphorylation [116]. Serum TNF concentrations were higher in active Crohn's disease patients relative to controls ($P < 0.01$) [117] and were negatively correlated with lean body mass ($r2 = 0.33$, $P = 0.023$) [117]. Data from this laboratory evidenced a trend for elevated plasma TNF concentrations in anabolically resistant paediatric Crohn's disease with mixed disease activity ($P = 0.078$) and reduced appendicular muscle mass relative to healthy controls ($P = 0.052$) [90]. These associations in active disease are likely indirect, and due to negative effects of inflammation on muscle mass and quality.

Despite the clear association between inflammation and active disease fatigue, most patients continue to report increased fatigue perception despite acheiving remission [57] [35] [118]. Quiescent patients with reduced concentrations of plasma TNF-alpha ($P = 0.002$) and increased IL-10 ($P = 0.01$) [88] continue to report signiciant fatigue symptoms compared to healthy controls. Consistently, other available data show plasma IL-12, IL-8 and IL-5 to be comparable between a cohort of fatigued and non-fatigued IBD patients with quiescent disease [119].

Inflammation is clearly an aetiological factor in active disease fatigue. However, the persistence of fatigue perception in patients with well-controlled disease and normal inflammatory markers refute the hypotheses that subclinical inflammatory burden contributes to fatigue aetiology. Clearly, other factors must predominate in quiescent disease fatigue.

### 2.4.3 IBD Malnutrition and Obesity

Malnutrition is common in IBD, up to 75% of hospitalised Crohn's disease patients are malnourished, and 50% are in negative nitrogen balance [120].This likely relates to classic symptoms including abdominal pain, vomiting and diarrhoea [121] which contribute to general feeling of malaise and loss of appetite. Other disease factors further influence malnutrition. Inflammation is linked to anorexia [122] , whilst altered eating behaviour due to hospitalisation and self-imposed dietary restrictions implemented to control GI symptoms [123, 124] limit dietary intake.

Due to malnutrition, several micronutrient deficiencies associated with fatigue development [89] such as iron, vitamin D [125-127], vitamin B12 [128] and thiamine [129] are common in IBD. However, experimentally it is inherently difficult to demonstrate a causal link between these micronutrient deficiencies and fatigue development. The next section of this chapter will provide a brief overview of these micronutrient deficiencies and their potential contribution to fatigue aetiology.

### 2.4.4 Anaemia

Anaemia prevalence is reported at 24% in IBD [130]. 27% of Crohn's disease and 21% of UC patients suffer from anaemia, which occurs due to blood losses and reduced iron absorption [131]. The prevalence of iron deficiency anaemia is reported at 20% in IBD [132] whilst iron deficiency in the absence of anaemia is reported at 37% [133]. Iron supplementation is associated with a reduced perception of fatigue burden in healthy volunteers with non-anaemic iron deficiency, but supplementation does not impact upon objective performance [134]. Although supplementation does target fatigue perception and muscle function in the elderly [135]. Self-reported fatigue perception does not differ in IBD patients with and without iron deficiency. This suggests no association between iron deficiency and fatigue perception when

assessed independently of anaemia [136]. Although in a cohort of 140 IBD patients, haemoglobin concentrations were weakly correlated to chronic fatigue perception in 20 UC patients with quiescent to mild or moderate disease ($\beta = -0.247$, $P = 0.014$) [37]. This implicates anaemia in the increased fatigue perception reported in UC. Furthermore, iron deficiency is associated with increased fatigue perception and premature exercise fatigue in the elderly, where iron supplementation increases exercise capacity [135]. There is a need to establish whether this association between iron deficiency and exercise fatigue [135] is also present in IBD, as this would represent a readily accessible treatment target.

### 2.4.5 Vitamin D

Vitamin D deficiency is associated with adverse effects on IBD disease course [137]. The prevalence of vitamin D deficiency is reported at 27% in Crohn's disease and 15% in UC [138]. No association was reported between vitamin D deficiency (<50 nmol/L) and fatigue perception in a cohort of 405 IBD patients [139]. However, vitamin D targets skeletal muscle via genomic [140] and cell surface vitamin D receptors with roles in calcium metabolism [141], [142] and myocyte proliferation [127] [126, 143]. In the elderly, vitamin D deficiency is associated with reduced muscle function [144], which can be targeted with vitamin D supplementation [145]. Consistently, sarcopenia is commoner in paediatric IBD patients who are vitamin D deficient [146] and cholecalciferol substitution improves muscle function in this cohort [147]. Low serum vitamin D3 (<50 nmol/L) is also independently associated with a greater rate of knee extensor fatigue in quiescent adult Crohn's disease [88] with atrophy of the vastus lateralis relative to healthy controls [99]. In the same subjects, Vitamin D3 was less in Crohn's disease patients with reduced active to total AKT phosphorylation (i.e. attenuated hypertrophy signalling). This could indicate a link between vitamin D deficiency and premature exercise fatigue in IBD.

### 2.4.6 Cobalamin / B12

Vitamin B12 has roles in nervous system function [148] and deficiency is commoner in Crohn's disease than in UC (18.4% vs 5%) [149] most likely due to the ileal location of Crohn's disease [150] and subsequent resection in a large proportion of cases. Fatigue perception is greater in stroke patients with a lacunar infarct and B12 deficiency relative to those without ($P$ = 0.01) [151] suggesting an association between B12 deficiency and fatigue. However, B12 supplementation does not target fatigue symptoms in patients with a lacunar infarct [152], IBD or IBS [153]. These collective findings suggest that B12 deficiency is not an aetiological factor in quiescent IBD fatigue.

### 2.4.7 Thiamine

Thiamine uptake occurs in the jejunum and is dephosphorylated by thiamine diphosphokinase to produce thiamine pyrophosphate [154] which serves as an enzymatic cofactor of three ketoacid dehydrogenases including pyruvate dehydrogenase, α-ketoglutarate dehydrogenase and branched chain α-ketoacid dehydrogenase. Thiamine deficiency is linked to the development of premature fatigue in IBD due to the association between thiamine deficiency, defective skeletal muscle pyruvate oxidation [155], and inhibition of mitochondrial ATP synthesis. High-dosage thiamine treatment improves fatigue symptoms in quiescent IBD patients, [68, 156] however the effect on exercise fatigue has not been characterised.

### 2.4.8 Obesity

The body mass of IBD patients is increasing [157]. Obesity is now a recognised as a metabolic comorbidity in IBD, with prevalence rates reported between 20% and 40% [158]. High visceral fat mass is associated with worsened IBD course [159] and postoperative complications [160]

including disease reoccurrence [161]. Likewise, the metabolic abnormalities that often accompany obesity, such as type II diabetes, negatively influence the course of IBD [162]. In the general population, obesity is associated with premature fatigue development [163].Deficits in peripheral muscle strength [164, 165], anaerobic task performance [166] and the resistance to exercise fatigue [165] are well established in obese individuals when compared to non-obese control volunteers. Consistently, targeting obesity is associated with improvements in the perception of fatigue and exercise fatigue [167]. In obese IBD patients, eight-weeks of concurrent exercise training intervention decreased total body fat percentage ($P < 0.001$), increased whole-body lean mass ($P < 0.0001$) and increased estimated $VO_2$ peak ($P = 0.03$) [168].

### 2.4.9 Fuel and protein metabolism

Hepatic and skeletal muscle glycogen stores represent the total body store of carbohydrate which is essential for sustained submaximal exercise performance [169]. Whilst there are no data available on glycogen content in IBD patients, both carbohydrate intake [57] [170] and whole-body glucose uptake and oxidation during an hyperinsulinaemic-euglycaemic clamp are consistent in IBD patients relative to healthy control volunteers [171]. However, hyperinsulinemia is reported in active and quiescent disease states [172] which is attributable to increased $\beta$ cell function [172]. Although this results in elevated HOMA index values (i.e., increased insulin resistance), available data suggest this is a potentially protective factor against disease reoccurrence [172].

Skeletal muscle insulin sensitivity has been shown to be consistent to age-matched controls in both paediatric [90] and adult [173] IBD cohorts. However, forearm glucose net uptake is blunted in paediatric Crohn's disease in remission relative to patients with active disease [90]. This may suggest greater muscle insulin resistance in quiescent IBD but requires further

investigation. Available data on energy expenditure in IBD suggest that malnutrition is not the result of hyper metabolism. Resting energy expenditure has been shown to be unchanged in IBD [174]. However, other Crohn's disease cohorts demonstrate minor elevations in resting energy expenditure [174], which remain when normalised to fat free mass. However, substrate metabolism is altered in IBD. Lipid oxidation has been shown to be increased and carbohydrate oxidation decreased in both the fasted and postprandial state in IBD patients [175]. These metabolic abnormalities are likened to a starvation phenotype and are potentiated in active IBD [174]. Enteral feeding has been shown to normalise substrate oxidation in IBD [174] and increase whole-body protein turnover [176]. Pharmacological therapy also further influences substrate oxidation. In paediatric Crohn's disease patients with active disease, infliximab treatment has been shown to reduce postprandial carbohydrate oxidation whilst simultaneously increasing lipid oxidation during a parenteral feeding intervention [177]. Similarly, the initiation of infliximab therapy reduces whole-body protein turnover in IBD patients [178]. This can be targeted with concurrent parenteral nutrition infusion, which improves protein balance relative to the fasting state both pre and post infliximab [178]. Infliximab treatment has also been shown to target IBD sarcopenia. Patients with active disease increased quadriceps strength (185 Nm vs 214 Nm, $P = 0.002$) and muscle volume (1505 $cm^3$ vs 1569 $cm^3$; $P = 0.010$) relative to pre-treatment baseline values [179].

Corticosteroid treatment increased whole-body protein breakdown in paediatric Crohn's disease [180] and has also been shown to increase postprandial protein oxidation in female Crohn's disease patients. This was concomitant to an increased carbohydrate oxidation and suppression of lipid oxidation in the fasting and postprandial states [181]. Thus, corticosteroid prescription negatively impacts substrate metabolism in IBD.

### 2.4.10 IBD Sarcopenia

Sarcopenia is now a recognised comorbidity in IBD [182]. Sarcopenia has a multifactorial aetiology including hormonal factors and attenuated anabolic signalling [183], physical inactivity [184], motor unit remodelling [185], peripheral muscle deconditioning [186], increased adiposity [187] and altered muscle phenotype [188]. The prevalence of sarcopenia is reported at 42% in IBD [182] and is associated with adverse clinical outcomes [189, 190] including the need for surgery [191]. Data from this laboratory have evidenced anabolic resistance to protein feeding in Paediatric Crohn's disease patients with mixed disease activity [90] but this did not occur in adults with active Crohn's disease [173]. Relative to healthy controls, fatigued patients with quiescent disease report heightened fatigue perception and demonstrate premature exercise fatigue [88] have lower serum insulin-like growth factor-1(IGF-1) concentrations [88] compared to a healthy control cohort. Consistently, thigh muscle cross sectional area was reduced (14%, $P = 0.055$) in a cohort of quiescent patients with attenuated hypertrophy signalling relative to healthy controls [99]. Atrophy of the gastrocnemius has been reported in active IBD relative to healthy control volunteers (3246 ± 417 vs 4415 ± 129 $\mu m^2$, $P = 0.01$) [117] alongside an altered muscle phenotype charachterised by a loss of MHCI and a concomitant upregulation of MHCIIa/MHCII proteins. This could indicate differential atrophy of MHCI fibres. i.e. a greater loss of MHCI fibre area, such that the biopsy sample becomes occupied by a greater proportion of MHCIIa/IIb fibres. Although the authors interpreted this as a shift from a slow oxidative, to a fast-fatigable phenotype in Crohn's disease. Which could be linked to the development of premature exercise fatigue in IBD.

When fatigued IBD patients with quiescent disease were stratified based on their phosphorylated: total Akt ratio, inflammatory markers were comparable between the high and low ratio groups, implicating factors other than inflammation in the attenuation of anabolic

signalling proteins [99] and premature exercise fatigue [88]. For example, serum IGF-1 concentrations were reduced by 37% in Crohn's disease and was associated with exercise fatigue during repeated maximal isometric thigh extensions (serum IGF-1<20 nmol/L,OR 64.72 [1.19, 3529], $P = 0.04$) [88]. IGF-1 acts upstream of the mTORC1 hypertrophy signalling pathway [192], and the reduced concentrations may represent a substrate for the attenuation in anabolic signalling reported in IBD and atrophy of skeletal muscle. Additionally, accelerometer based physical activity was consistent between Crohn's disease and healthy controls [88]; although, patients with a low phosphorylated: total Akt ratio were less active than the patients in the high ratio group ($P = 0.009$). Which is consistent with disuse atrophy [184]. Physical inactivity is reported across the lifespan in IBD [91] and may be linked to the molecular dysregulation, muscle atrophy [99] and premature exercise fatigue [88] reported in IBD. Surgery rates are decreasing in IBD [193] although the inactivity and deconditioning associated with hospitalisation should be considered in fatigue aetiology given the negative effect on skeletal muscle health [194, 195]. Aerobic exercise capacity is reduced to a greater extent in IBD patients with extensive small bowel resection relative to non-resected patients [95]. Whilst a greater magnitude of disease burden undoubtedly contributes to this loss of function, the inactivity associated with surgery, hospitalisation and general chronic illness is likely a significant contributor.

The collective findings relating to disease burden [117], anabolic dysregulation [99] reduced protein nutrition [170], altered substrate metabolism [178, 180] and physical inactivity [91] all pose significant risk for sarcopenia development in IBD and may be additive in effect. The absence of interventions known to successfully target inactivity-induced muscle decline [196, 197], including exercise training [195] and nutritional intervention suggests that sedentary IBD patients, particularly those with greater disease activity requiring hospital treatment during relapses, are unlikely to recover the deficits in muscle quality. This is a particularly important

consideration given the chronic relapsing nature of IBD; where patients experience cyclical disease activity with intervening periods of remission. This cyclical disease activity is likely to accentuate these risk factors for the development of sarcopenia (e.g. repeated disease flares in the absence of appropriate rehabilitation in intervening periods of remission). This is a particular concern given the earlier mean age of onset of IBD relative to other chronic diseases, potentially resulting in lifelong relapsing remitting disease activity. This is consistent with the muscle atrophy, premature exercise fatigue and continued perception of increased fatigue [88] reported in well controlled IBD. This highlights the potential for exercise training and nutritional interventions during periods of disease remission to improve overall health status prior to potential future disease relapse and the accompanying physical in-activity associated with general illness and hospitalisation.

### 2.4.11 CNS changes in IBD fatigue

Whilst fatigue domains have not been considered as primary outcome variables in neuroimaging experiments in IBD, available data on brain structural changes suggest an involvement in fatigue aetiology. The atrophy of grey matter in the right supplementary motor and reduced axonal diffusivity in the right corticospinal tract [198] are consistent with the perturbed neuromuscular function in IBD [87] and could represent neural alterations that modulate premature exercise fatigue [86].

Fatigued Crohn's disease patients with quiescent disease demonstrate grey matter atrophy in the superior frontal gyrus relative to healthy controls [199]. The superior frontal gyrus is involved in cognitive processing [200], which may be linked to altered emotional processing in IBD [201]. This could influence fatigue perception given the strong influence of psychological factors across fatigue domains. Grey matter cerebral blood flow is also increased in fatigued Crohn’s disease patients [199]. The mechanisms modulating these structural brain

alterations and the clinical significance of such changes across chronic disease fatigue [202] are essentially unknown. Inflammation is often implicated in these morphological and haemodynamic alterations [201, 203]. Although there are currently no causal data to support this. Cytokines can cross the blood brain barrier [204] to stimulate inflammatory signalling within the CNS. The atrophy of grey matter seen in IBD may be explained by the neurotoxic effects of pro inflammatory cytokines [205] [206]. Further, macrophages are known to secrete nitric oxide [207], which could be linked to the hyper perfusion of grey matter reported in fatigued Crohn's disease patients [199].

### 2.4.12 Psychological factors

Psychological disorders are common in IBD [208]. There is a considerable overlap in the symptomology of anxiety, depression and fatigue [49]. Clinical factors such as Pill burden (i.e. a reduced adherence or difficulties in adhering to medications [209]) due to the chronic lifelong care required in IBD is associated with treatment fatigue. Indeed, 30% of IBD patients reported pill burden as a reason for non-adherence to medication [210]. This likely influences overall fatigue perception. Psychological factors influence disease course [211, 212] and fatigue symptomology [63, 111]. Quiescent IBD patients with a self-directed personality [103] and a higher sense of coherence [213] self-report lower fatigue burden than other patients. This suggests a general role of psychological state in fatigue perception, which is consistent with recent models of fatigue. Similarly, psychological state heavily influences exercise performance. The provision of verbal encouragement has an ergogenic effect during exercise, as does listening to music. The phenomenon of end-spurt capabilities, where healthy volunteers remain capable of significant, transient elevations in power output at the end of task performance, even following aerobic task failure, provides clear evidence for an important role of psychological state on exercise fatigue [24, 25]. The relationship between fatigue perception

and exercise fatigue is not well defined and may differ across chronic disease. Self-reported fatigue perception on the physical facet of the FIS scale positively correlated with the gradient of force decline during repeated isometric knee extensor contractions [88], although as previously mentioned, the relevance of this chosen mode of fatigue assessment is questionable.

### 2.4.13 Treatment-related factors

Increased fatigue perception is a reported following the prescription of many medications used in the treatment of IBD. Immunomodulators such as azathioprine are associated with increased fatigue perception in Crohn's disease patients. Similarly, the initiation of thiopurine therapy is known to stimulate marked fatigue symptoms, which transiently resolve upon cessation of treatment [48]. The use of Anti-TNF treatment at baseline has been linked to a greater severity of fatigue perception in IBD [214] whilst the cessation of biological therapy was associated with a reduction in the magnitude of fatigue perception [63]. In contrast, 12 weeks of conventional therapy consisting of 5-aminosalicylates,corticosteroids and/or thiopurine successfully reduced fatigue perception in 82 newly diagnosed UC patients [215]. Likewise, treatment with both infliximab [35] and adalimumab [69] have also been shown to reduce fatigue perception when assessed as secondary outcome variables. These contrasting data linking IBD therapies to the development of increased fatigue perception [214] suggest that the disease activity in patients is a confounding factor, as appose to a specific drug-related effect.

Glucocorticoid treatment targets inflammation during active disease [216] and has been shown to supress the inflammation induced stimulation of E3 ligases in rodent skeletal muscle [217]. However, glucocorticoid treatment is associated with higher fatigue perception in IBD [63] and has been shown to negatively influence protein turnover in paediatric IBD [180] and substrate oxidation in female IBD patients [181]. The catabolic effects of glucocorticoids are mediated by the E3 ubiquitin ligase muscle RING finger protein 1 (MuRF1) dependent degradation of

skeletal muscle contractile proteins [218] and have been demonstrated to inhibit both muscle metabolic [219] and contractile function [220]. Consistently, body cell mass is negatively correlated with cumulative prednisolone dose ($\varphi = -0.318$, $P = 0.011$) in female IBD patients [89] which is consistent with the muscle weakness documented in female IBD, that was also of a greater magnitude than male Crohn's disease patients. Limitations in the experimental design of existing data prevent more resolute conclusions on drug factors relating to IBD fatigue.

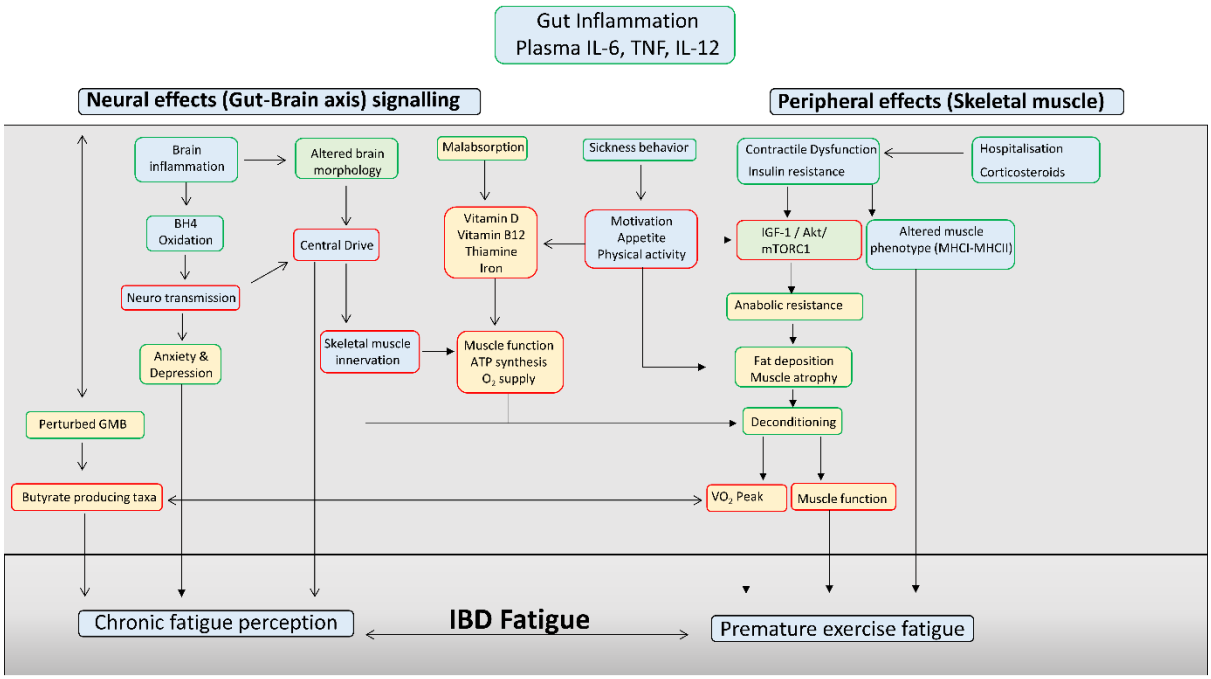


***Figure 2-1***. *Theoretical schematic of IBD fatigue aetiology taken from McGing et al. [1] Light blue boxes reflect active disease mechanisms. Light green boxes relate to available data in quiescent disease fatigue. Yellow boxes are co-occurring factors in both disease states. Green outline represents upregulated processes whilst red outlines represented downregulated processes. Closed arrows represent aetiological factors predominantly contributing to one fatigue domain. Open arrows represent factors that can contribute to either domain.*

## 2.5 Available treatment strategies for IBD fatigue

There are currently few evidenced base treatment strategies available for IBD fatigue. The next section of this chapter will review the effectiveness of the treatment strategies that have been trialled.

### 2.5.1 Pharmacological management

An early open label pilot study demonstrated complete regression of fatigue perception assessed by the CFS scale following 20 days of high dosage thiamine therapy in 83% of a small 12 patient cohort [156]. More recently, these findings were corroborated in an RCT of high dosage thiamine treatment, which successfully alleviated fatigue in forty quiescent IBD patients with persistent fatigue perception [68].

Currently available data on the efficacy of anti-TNF therapy on fatigue in IBD are inconclusive. Fatigue perception significantly reduced following 4 weeks of infliximab therapy when assessed as a secondary outcome using the MFI-20. Whereas a placebo infusion rapidly decreased fatigue scores, but returned to baseline levels within 2 weeks. [35]. Consistently, in a cohort of 499 Crohn's disease patients with moderate-to-severe disease activity, adalimumab therapy (40 mg every 2 weeks) significantly reduced fatigue perception when fatigue was assessed as a secondary outcome measure using the FACIT-F scale [69]. These experimental designs are unable to delineate the mechanisms driving the observed improvements in self-reported fatigue perception in these IBD patients. Given the relationship between disease activity and fatigue. [118, 221] It is likely that the observed reductions in fatigue burden were indirect, resulting from an improvement in disease activity. Infliximab treatment may also positively influence exercise fatigue as infliximab treatment has been shown to target sarcopenia in IBD, increasing both knee extensor volume and isokinetic strength in adult

Crohn's disease [179]. Despite these positive effects, biological therapy generally fails to improve fatigue burden in most IBD patients. In a cohort of 198 fatigued IBD patients, 28% remained classified as fatigued from their self-reported scores of fatigue perception following 54 weeks of biological therapy, despite reaching clinical remission (HBI ≤4 or SCCAI ≤2 and CRP <8 mg/L) [118]. These collective findings do not support the use of anti-TNF therapies solely for the treatment of IBD fatigue.

### 2.5.2 Psychosocial interventions

In line with the psychological contribution to fatigue aetiology, psychosocial interventions have demonstrated potential efficacy in targeting IBD fatigue. Stress management programmes led by a therapist or self-directed by the patient have reduced self-reported tiredness, assessed as a secondary outcome at 6- and 12-months follow-up in Crohn's disease patients using a Crohn's disease symptom diary [222]. Consistently, problem solving therapy (PST) and solution-focused therapy (SFT) have been shown to reduce self-reported fatigue perception by 60% and 85.7% following a 12-weekpilot intervention in Crohn's disease patients with inactive disease [67]. Subsequently, this proceeded a 12-week RCT in 98 fatigued patients with inactive Crohn's disease comparing SFT to care as usual in a sample of 98 fatigued Crohn's disease patients in remission [223]. SFT was shown to reduce fatigue perception relative to care as usual ($P < 0.001$). This effect remained consistent at 6-month follow-up ($P < 0.010$) but diminished at 9 months ($P = 0.610$). These positive effects were attributed to cognitive reappraisal and reduced self-perception of illness in the patients, which appears rationale given the psychological contribution to fatigue aetiology. The authors also attributed the diminished effect observed at 9 months to the absence of follow-up plans. Whilst this may be feasible, these interventions only target psychological facets of fatigue aetiology. The sole prescription of a psychosocial intervention therefore fails to target the many other physiological factors

contributing to fatigue. This may account for the transient effect observed on fatigue perception [223].

### 2.5.3 Physical activity interventions

Exercise training interventions have been shown to have a positive effect on both self-reported fatigue burden and premature exercise fatigue in chronic diseases such as MS and cancer, where heightened fatigue perception, sarcopenia, and deconditioning are common [224, 225].

Fatigue has not been considered as a primary outcome measure in response to exercise training in IBD. The aforementioned study from McNelly and colleagues compared the effect of increased physical activity and/or omega-3fatty acid supplementation on fatigue in a cohort of 52 IBD patients in remission. However, this produced inconclusive results as both interventions displayed inconsistent effects dependent upon the instrument used to assess fatigue perception [51]. More recently, six months of resistance exercise training in a cohort of 23 IBD patients was shown to reduce fatigue perception ($P < 0.005$) when assessed by the IBD fatigue scale. Increases in lumbar spine bone mineral density (BMD) ($P < 0.001$) and isometric elbow and knee extensor strength ($P < 0.001$) were also reported relative to a control group [226].

## 2.6 Contemporary issues in IBD fatigue research

The available data reviewed within this chapter highlights a fundamental lack of understanding of IBD fatigue aetiology. Whilst the available physiological data can indeed highlight probable aetiogical factors, such as deconditioning [41] (reduction in muscle strength and cardiorespiratory fitness), immune factors [42] and microbiota composition [43]. There is an absence of mechanistic data available. This prevents any resolute conclusions on IBD fatigue aetiology, which limits the identification and assessment of potential treatment options.

Reviewing treatment options that have demonstrated efficacy in similar chronic diseases that also present with sarcopenia, weakness, and fatigue is a rationale start point. This likely underlies the recent provision of exercise training interventions in IBD, which also includes fatigue perception assessment [226]. As targeting deconditioning with exercise training intervention has been shown to alleviate fatigue burden in cancer [225, 227], MS [224] and RA [228]. However, this approach lacks specificity. Although there are common correlates of fatigue aetiology, the fundamental difference in disease pathophysiology between these chronic diseases calls for the design and optimisation of IBD specific exercise training interventions to optimise the management of fatigue.

## 2.7 Non-invasive approaches to fatigue assessment

During this thesis, functional, $^{1}$H MRI and $^{31}$P MRS phenotyping experiments have been performed to comprehensively interrogate IBD physiology.

### 2.7.1 $^{31}$P Magnetic Resonance Spectroscopy to assess peripheral muscle quality

$^{31}$P MRS has been utilised to probe in-vivo muscle metabolism for four decades and provides a unique opportunity to non-invasively characterise high-energy phosphate metabolism. $^{31}$P MRS enables quantitation of $^{31}$P metabolites that can be expressed as metabolite ratios. Absolute metabolite concentrations can also be estimated by assuming the concentration of an internal reference (e.g. muscle ATP). Quantifying the recovery kinetics of $^{31}$P metabolites such as PCr during exercise recovery enables inference on muscle mitochondrial function. As the ATP utilised to resynthesize PCr via the creatine kinase reaction ($PCr + ADP + H^{+} \xleftrightarrow{CK} ATP + Cr$) is synthesised via mitochondrial oxidative phosphorylation [229]. This widely applied technique is able to characterise muscle oxidative capacity across the lifespan [230, 231], between training status [232], different muscle groups [233] and in chronic diseases

[234-236], including those with a high prevalence of fatigue [234, 237, 238]. $^{31}$P MRS has also been used to assess muscle metabolic adaptations following exercise training intervention in these diseases [239-241].

### 2.7.2 Cerebral and cardiac blood flow and cerebral oxygen extraction

Cerebral blood flow (CBF) is known to increase during low to moderate exercise intensities [242] to facilitate oxygen supply for neuronal metabolism [243, 244]. For example, the cortical activation during dynamic movement is associated with elevated blood flow to the supplementary motor area [245]. At increased exercise intensities (> 60% $VO_2$ peak) the accompanying hyperventilation and subsequent hypocapnia results in an uncoupling of CBF and neuronal metabolism due to cerebral vasoconstriction. Thus, CBF returns toward resting levels at these higher exercise intensities [242]. The continued increase in neuronal metabolism with increasing exercise intensity [242] is met by an increased uptake of lactate, glucose and oxygen [246].

The changes in CBF and oxygenation at low-moderate exercise intensities associates with neuronal activity [244, 245, 247]. Disturbances to these parameters on exercise are associated with the development of central fatigue [248]. Thus, the assessment of cerebral blood flow and brain oxygen metabolism during low-moderate intensity exercise is of interest in deconditioned cohorts presenting with fatigue. Previous data from University of Nottingham has demonstrated variations in CBF between active and non-active grey matter in healthy volunteers, that shows a linear response to exercise tasks at 30 and 50% $VO_2$ max. This points to a regulatory role of cardiac output and CBF in the coupling of regional cerebral blood flow and neuronal activity. Further data has characterised stepwise elevations in CBF during supine cycling exercise performed at 30 and 50% of supine $VO_2$ peak, which returns to baseline values during recovery from exercise. However, this CBF response was blunted in deconditioned

elderly subjects, presenting with a reduced $VO_2$ peak, relative to healthy young control volunteers [249]. These data are consistent with previous comparisons of cerebral perfusion between young and elderly individuals during sustained submaximal exercise, using transcranial Doppler ultrasonography [250]. The blunted CBF response observed on exercise in the elderly, relative to young healthy controls was partially attributed to lower $PCO_2$ concentrations in the elderly. Collectively these data suggest that cardiac output potentially modulates cerebral blood flow and thus brain neuronal activity during sustained aerobic exercise. This suggests that deconditioning (i.e. a reduction in $VO_2$ peak) may potentiate central fatigue during task performance, as perturbations in cerebral metabolism are implicated in reduced central drive to skeletal muscle [248].

## 2.8 Conclusion

Mechanistic studies are required to delineate the fundamental aetiological factors contributing to fatigue, prior to the optimisation of either physical training interventions or pharmacological treatments. The current prescription of exercise training intervention to IBD patients [251, 252] is based on indirect evidence from other chronic diseases with muscle decline [224, 225] where exercise training improves fatigue burden. Data generated from such interventional studies will not directly inform on IBD fatigue aetiology. The combined application of $^{1}H$ MRI and $^{31}P$ MRS phenotyping outlined in the next chapters will provide novel insights into quiescent IBD physiology and aid in the identification of modifiable fatigue correlates that can be directly targeted by IBD intervention.

## 2.9 Experimental aims

The aim of this thesis is to characterise quiescent IBD physiology via comprehensive functional, $^{1}H$ MRI and $^{31}P$ MRS phenotyping of Crohn's disease patients and healthy

volunteers. Furthermore, the developmental work undertaken during this thesis will outline experimental protocols within the exercise-MRI theme, that can be applied to other chronic disease presenting with sarcopenia and premature fatigue.

### 2.9.1 Central deconditioning

We aimed to assess cardiac output and brain vascular and metabolic responses across a low intensity, supine exercise task in Crohn's disease patients relative to healthy control volunteers.

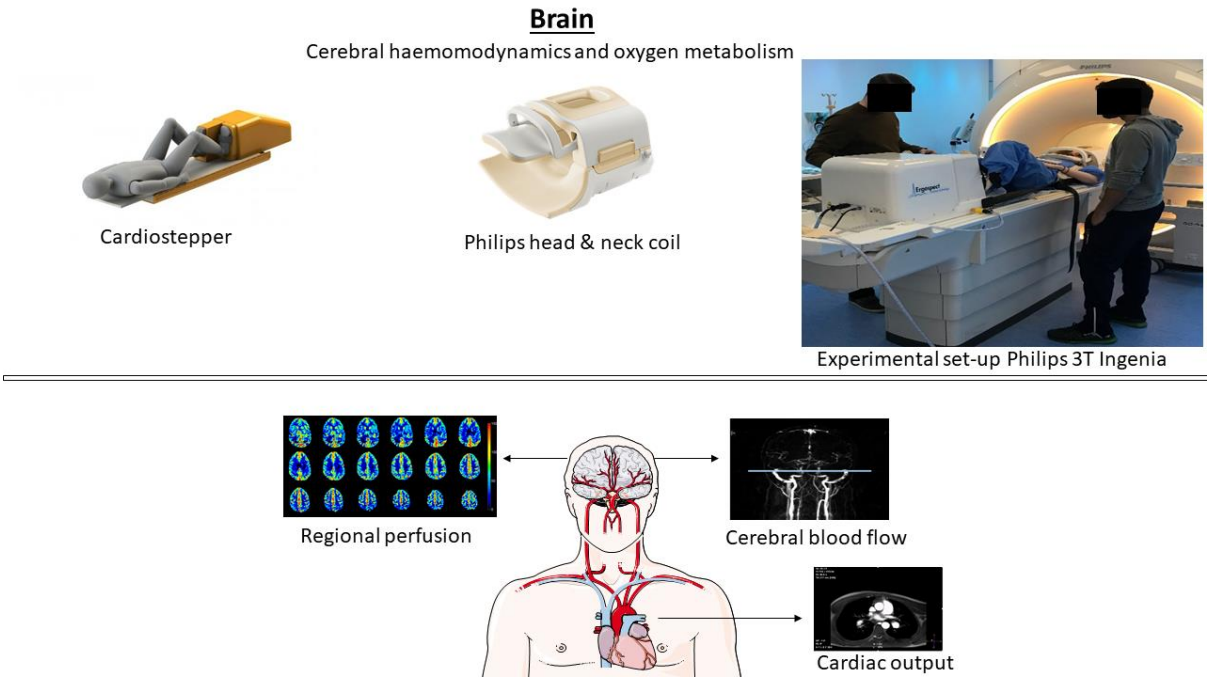


*Figure 2-2. Example of experimental $^{1}H$ exercise-MRI protocol to investigate central deconditioning in IBD fatigue.*

### 2.9.2 Peripheral deconditioning

We aimed to quantify the rate constant of PCr resynthesis ($k_{PCr}$) following ischemic plantar flexion exercise at a standardised relative exercise intensity as an indirect measurement of muscle metabolic quality.

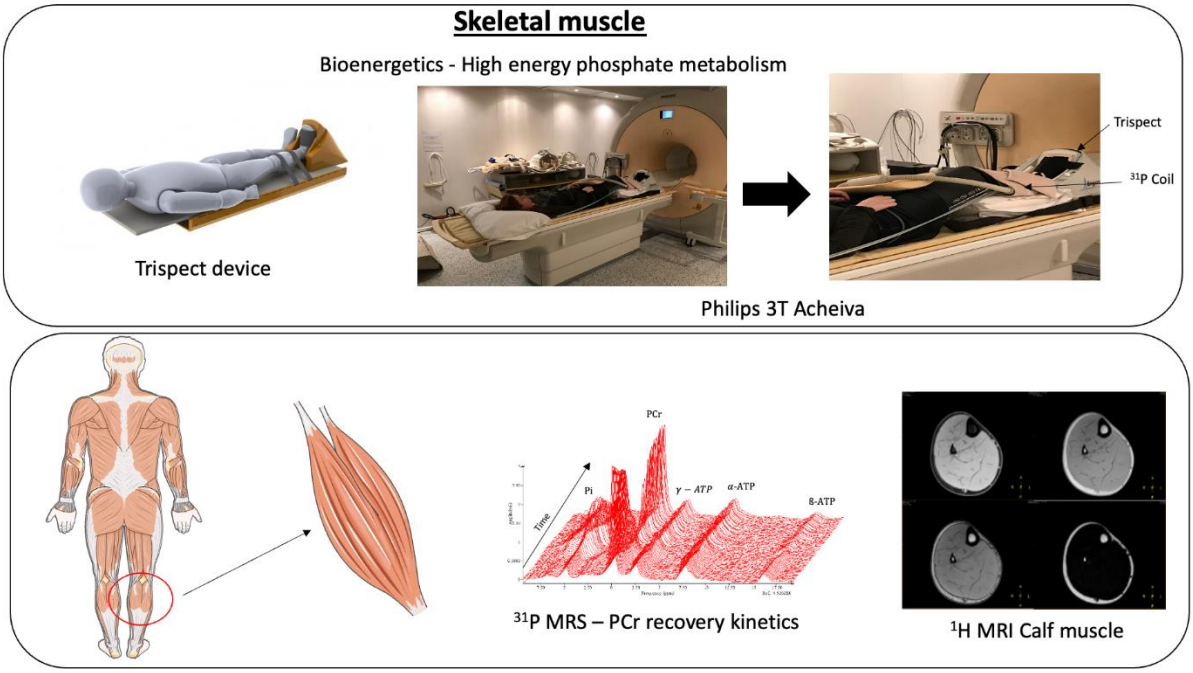


*Figure 2-3 Example of experimental $^{31}P$ MRS protocol to investigate muscle metabolic quality in IBD.*

These data will be collected together with functional laboratory measurements including $VO_2$ peak, skeletal muscle strength and fatiguability. It is likely that the development of premature exercise fatigue observed in IBD patients is at least in part attributable to peripheral deconditioning (i.e. a reduction in mitochondrial density) which is common in chronic disease [234, 237]. If IBD patients are found to have elevated fatigue perception and impaired muscle mitochondrial function relative to healthy control volunteers, it may suggest exercise training intervention could improve fatigue burden. Similarly, if IBD patients demonstrate premature exercise fatigue alongside deficits in the neurovascular measurements, targeting central

deconditioning via exercise training intervention with an aerobic component may improve fatigue burden.

## 2.10 Experimental protocol

### 2.10.1 Central assessment

Exercise fatigue mechanisms result from the interlinking of the cardiorespiratory system, skeletal muscle and the CNS [70]. Previous experiments from this laboratory using MRI exercise protocols have revealed alterations in cerebral haemodynamic and oxygen metabolism across the lifespan [249, 253]. MRI based measurement of global and regional cerebral blood flow provide a non-invasive, indirect measurement of cerebral haemodynamics, serving as a proxy of brain neuronal activity. We aimed to apply these measurements to IBD patients in order to assess central deconditioning. If cardiac output and cerebral blood flow is altered in IBD patients during low intensity continuous exercise, this may be reversible via exercise training or pharmacological intervention.

### 2.10.2 Peripheral assessment

$^{31}$P MRS is a well-established method used to quantify high energy phosphate metabolism in skeletal muscle (see methods chapter). There is ample evidence of peripheral muscle dysfunction in IBD patients. Both maximum strength [86] and fatiguability [86, 88] of peripheral skeletal muscle has been shown to be impaired relative to healthy control cohorts. It is probable that the premature exercise fatigue is linked to a reduced mitochondrial density (i.e. peripheral muscle deconditioning) or an inherent mitochondrial deficit. However, there is currently no evidence to support this. Dysregulation in anabolic signalling proteins have been identified in vastus lateralis biopsy samples in IBD [99] although there is no data available on

mitochondrial content, only functional measurements of exercise performance where premature fatigue is common [86, 96]. The application of $^{31}$P MRS sequences during within-bore plantar flexion exercise will enable indirect quantitation of muscle metabolic quality, by tracking the resynthesis rate of skeletal muscle PCr during exercise recovery. Skeletal muscle ATP concentrations remain relatively constant during exercise [254]. This is achieved by the regulation of oxidative and non-oxidative ATP synthesis. The relative contribution of these energy systems and associated rate of metabolite accumulation is determined by contractile intensity [255]. Premature exercise fatigue in chronic disease is linked to both reduced mitochondrial function (mitochondrial mass or inherent functional loss) and impaired skeletal muscle perfusion [256]. Since muscle phosphocreatine (PCr) resynthesis is wholly mitochondrial dependent [257] and thus completely supressed under ischaemia [258], assessing post-exercise PCr recovery kinetics after reinstating limb blood flow, where oxygen delivery is not a limiting factor, allows for non-invasive quantitation of muscle mitochondrial function.

### 2.11 Impact statement

As performed in number of recent experiments investigating IBD fatigue aetiology [41-43], the original IBD Fatigue study protocol aimed to stratify recruited Crohn's Disease patients into a fatigued and non-fatigued sub-group. It was proposed that this would be performed using the MFI-20 instrument (see section 3.2.2 for detailed description) according to previously defined cut off used in IBD cohorts [13] where the fatigue scores related to the 75$^{th}$ percentile of MFI-20 scores in the general population [40]. However, a significant amount of time was dedicated to resolving the hardware issues described in the development chapter. This was integral to the experiment in order to validate the instruments and experimental protocols prior to commencing the study. This elongated developmental period delayed the experimental data

collection by circa 18 months. Concomitantly, an extension to the registered study period of the PhD was granted. It was anticipated that this would enable completion of the originally proposed study protocol (i.e. recruitment and completion of 48 total subjects split between health controls, fatigued and non-fatigued CD patients). However, following the initial outbreak of the COVID-19 pandemic, the IBD Fatigue study was suspended by the University of Nottingham in March 2020 and was not given permission to re-start until July 2021. Whilst the University granted a phase 1 COVID related extension, this additional funding period expired 6 months before the study was given permission to re-start. Upon re-start of the project, the PhD submission deadline remained at January 2021 and was unlikely to be granted further extension requests as the PhD funding had already expired.

A supervisory meeting took place in September 2021 to determine the minimum sample size required in order to adequately complete the study in the context of the remaining PhD duration, funding constraints and statistical power. Available $^{31}$P MRS data was reviewed as interim data analyses had revealed the only differences in any physiological outcome measurements were the PCr recovery kinetics.

After reviewing the available data together with consideration of the maximum rate of volunteer recruitment, together with data analysis and thesis writing, it was evident that achieving the original N=48 sample size was unattainable given current time and funding constraints. Based on sample size calculations performed at the time (see the methods section of the experimental chapters) it was decided by the supervisory team that the best approach would be to collapse the current stratified patient subgroups to perform a HV vs CD comparison.

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# Chapter 3. Validation of MR compatible ergometers and development experiments.

### 3.0 Introduction

This chapter outlines the development of the protocols to perform exercise studies within the MR scanners. Traditionally, MR scanning techniques are performed under resting conditions. However, the provision of physiological stress (e.g. exercise stimulus) and measurement of metabolic response during imaging can reveal key information on organ function that would otherwise be masked under traditional experimental conditions. Here the set-up of the Ergospect ergometers (https://ergospect.com), the Cardiostepper and Trispect modules, are outlined. These devices are used to stimulate exercise stress within the MR bore which enables non-invasive, dynamic assessment of organ physiology.

The development of MR technology has proven to be an invaluable tool for probing multi-organ physiology and is utilised for a multitude of clinical and research applications. MRI provides excellent soft tissue contrast at high spatial and temporal resolutions independent of ionizing radiation exposure or invasive biological procedures. Anatomical MRI can be applied across multiple organ systems for imaging the musculoskeletal system [1] and brain injury [2] as well as neurological disease diagnoses [3], evaluation of treatment response [4] and comprehensive physiological phenotyping of disease pathophysiology [5].

Advanced MRI methods enable the quantification of multi organ processes including cardiac, cerebral and renal haemodynamics and *in-vivo* metabolism of the brain [6], liver [7], skeletal [8] and cardiac [9] muscle. These methods have become an invaluable physiological research tool enabling non-invasive characterisation of pathophysiological alterations.

Traditionally, MRI/MRS approaches have been used to obtain data on morphometry, haemodynamics, and cellular metabolism under resting state conditions. However, in certain clinical scenarios, the collection of resting state physiological data can be of limited usefulness. For example, heart failure patients demonstrate comparable resting state phosphate ratios and intracellular pH in skeletal muscle relative to healthy control subjects. However, the increase in Pi/PCr and intracellular acidosis following a within-bore exercise task is significantly greater in heart failure patients [10] evidencing impairments in energy metabolism that are not apparent under resting conditions. Recent developments in hardware and multimodal MR ergometers [11-13] enable dynamic MR assessment of numerous organ systems as will be outlined in this chapter. Within-bore exercise also provides an attractive alternative to common pharmacological interventions, such as Dobutamine stress testing [14] which is commonly used to evaluate cardiac function but is invasive and carries significant risk of side effects.

In Section 3.1 of this chapter, the development and optimisation of the Cardiostepper is described along with the set-up of the MRI protocol to assess central deconditioning through assessment of cardiac output and brain vascular and metabolic response to exercise (Figure 3-1). This work is performed on the 3T Ingenia, a wide bore MR system with a 70 cm scanner bore.

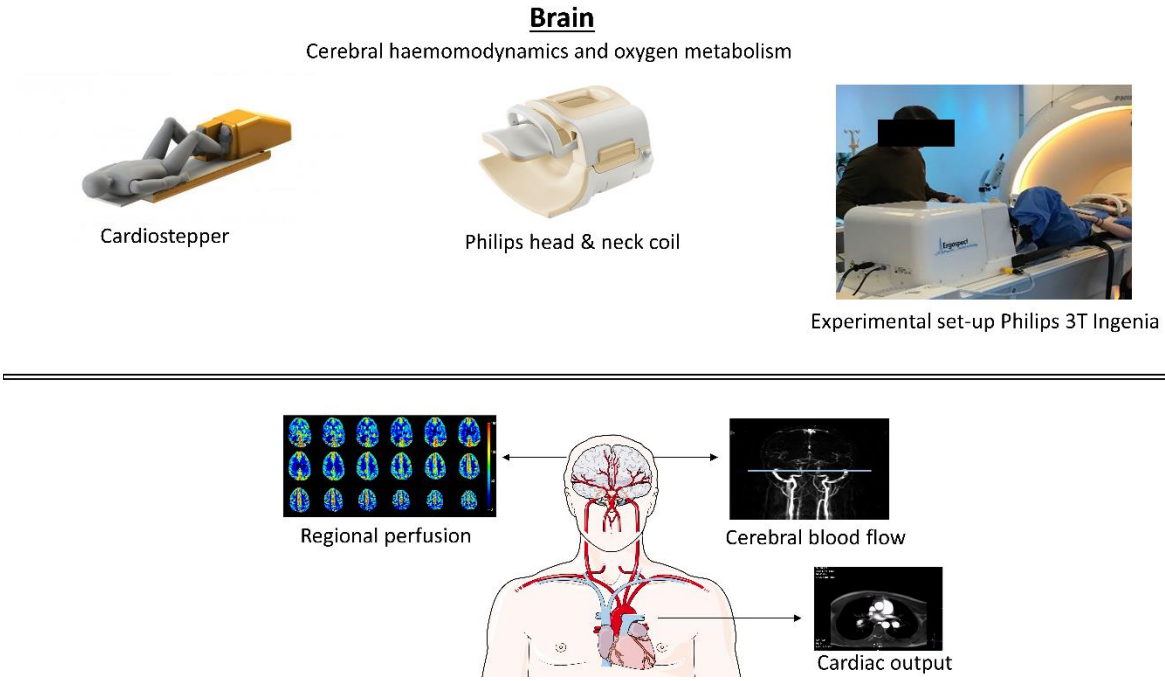



***Figure 3-1****. Schematic of Cardiostepper module to interrogate central deconditioning on the 3T Philips Ingenia scanner through MRI measures of the heart and brain using a head & neck coil.*

In Section 3.2 the set-up and use of the Trispect to assess peripheral deconditioning through a metabolic study using phosphorus MR spectroscopy ($^{31}$P MRS) to quantify energy phosphate metabolism in skeletal muscle is described (Figure 3-2). This work is performed on the 3T Achieva system, a 60 cm narrow bore MR scanner, but this is a multinuclear enabled system allowing the study of phosphorus ($^{31}$P) metabolism.

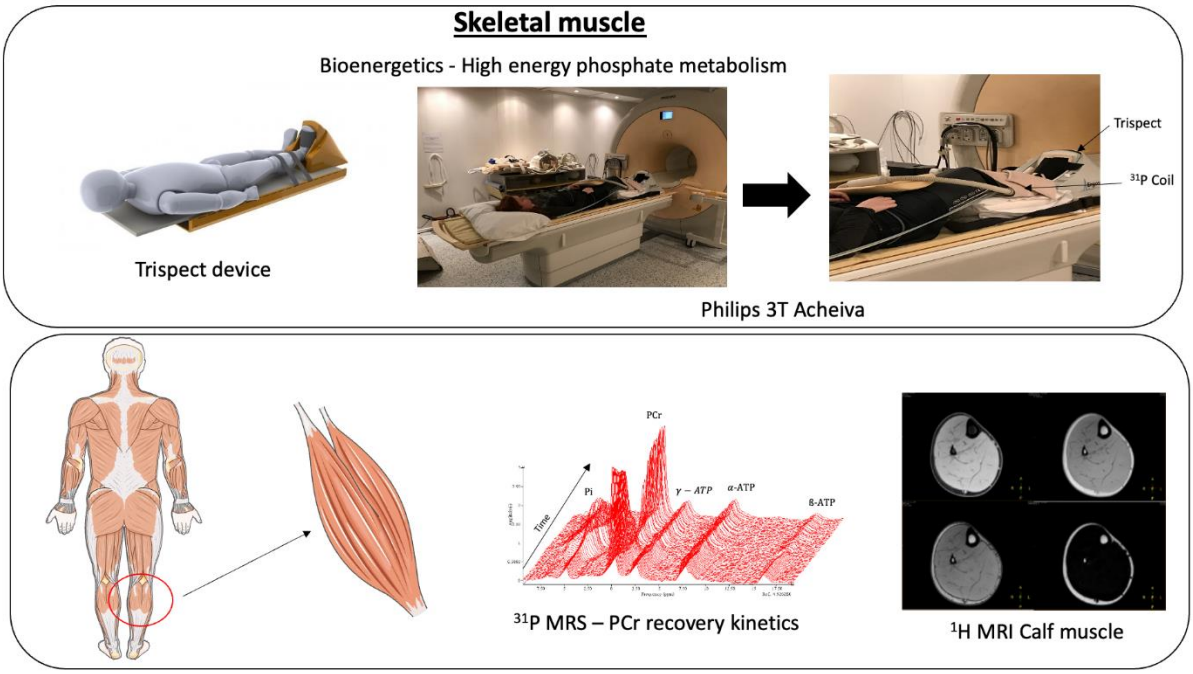



***Figure 3-2.*** *Schematic of Trispect module used to interrogate peripheral deconditioning on the 3T Philips Achieva scanner through $^{31}P$ MRS and $^{1}H$ MRI measures of the muscle.*

Prior to this work, MRI exercise studies had been performed at the University of Nottingham using the Lode cycle Ergometer (Lode, Germany) to investigate metabolic correlates of ageing [15]. However, this system had biomechanical limitations (i.e. knee contact with exterior of the scanner during cycling exercise) as well as producing difficulties with excessive motion artefacts associated with cycling exercise. Thus, for this study a Cardiostepper module for supine exercise within the magnet was purchased, with the model chosen to be a 7T-compatible version to allow future studies on the 7T Philips system at the University of Nottingham. The experimental laboratory set-up at the David Greenfield Human Physiology Unit (DGHPU, University of Nottingham) was modified to enable supine exercise experiments to be performed using the newly purchased Ergospect Cardiostepper module (Ergospect, Innsbruck, Austria https://ergospect.com/). Alongside this, an Ergospect Trispect module was also purchased for assessment of peripheral deconditioning (see Section 3.2), with both systems

having the same control units, as shown in Figure 4-3. The overall goal of the work outlined in this chapter was to develop and validate experimental protocols for these two Ergospect modules. Initial measures were collected to enable incremental exercise testing to establish supine $VO_2$ peak and peak aerobic power on the Cardiostepper module, and on the Trispect module in order to establish maximum voluntary contractions (MVC) in a supine position. Studies were then performed to enable within-bore exercise experiments during cardiorespiratory and resistance exercise at relative intensities within the MRI scanner at the Sir Peters Mansfield Imaging Centre (SPMIC, University of Nottingham).

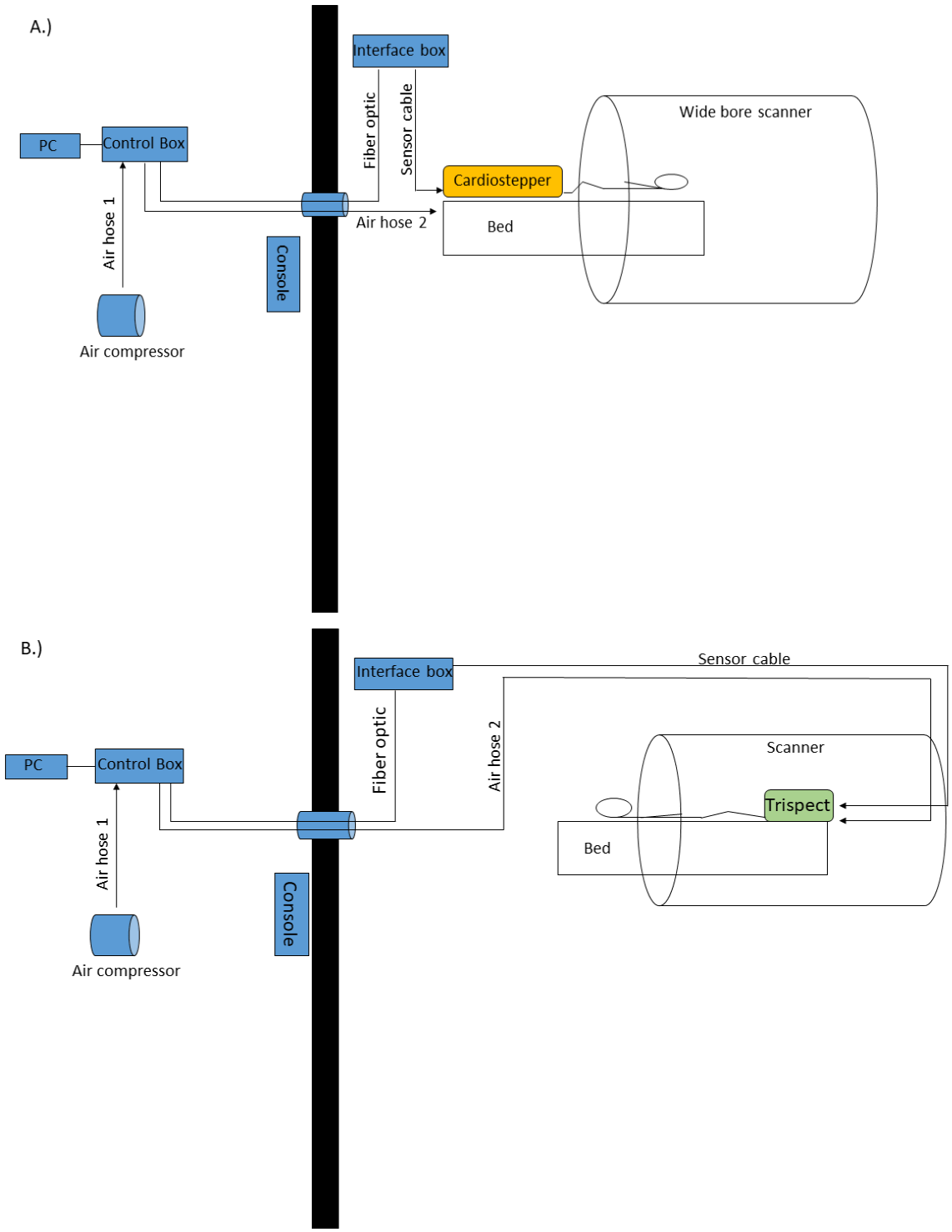



***Figure 3-3****. Schematic of experimental set up with Ergospect devices shown for a) the Cardiostepper module and b) the Trispect module.*

### 3.1 Assessing Central Deconditioning using the Cardiostepper

This section outlines the development of the Cardiostepper in conjunction with Ergospect, and the MRI protocol set-up to study central deconditioning.

#### 3.1.1 Evaluating the 7T Compatible Ergospect Cardiostepper

To validate the efficacy of the Ergospect Cardiostepper to establish a supine $VO_2$ peak, four healthy volunteers completed a supine incremental exercise testing protocol at the David Greenfield Human Physiology Unit (DGHPU) (Table 3-1.).

| Experiment | Subject | No. | Age | Height (cm) | Body mass (kg) | BMI |
|---|---|---|---|---|---|---|
| Development | Mixed cohort | 4 | 36 ± 7 | 171 ± 6 | 71.1 ± 9.1 | 24 ± 2 |
| | Female | 2 | 39 | 166.5 ± 4.9 | 65.6 ± 8.3 | 24 ± 2 |
| | Male | 2 | 33 ± 11 | 175 ± 1.4 | 76.50 ± 7.8 | 25.0 ± 2 |
| Repeatability | Male | 1 | 25 | 176 | 82 | 27 |

***Table 3-1:*** *Demographic of healthy volunteers who performed testing of the 7T Cardiostepper*

Volunteers underwent basic health screening measures and an ECG prior to exercise studies. For the testing, the 7T-compatible Ergospect Cardiostepper was mounted and secured onto a *Phillips* MRI bed on a custom-made support frame (Fig. 3-4 (I.). Volunteers were positioned on the Cardiostepper module using a waist coat which prevents excess motion and fixes the knee angle during supine exercise (Fig 3-4, III-IV.) A 28 mm turbine containing a sampling line was connected to the volunteer's face-mask for continuous breath-by-breath online gas analysis (Cosmed, Rome, Italy). Volunteers were required to step to the beat of a metronome (70 steps per minute) and began stepping at a 50W workload. All exercise rounds were of 3-minute duration and the workload was increased by 20-25W at the end of each 3-minute round.

Volunteers were encouraged to exercise until volitional exhaustion. Volunteer rate of perceived exertion (RPE), heart rate (HR) and step frequency were manually recorded during the final 30s of each 3-minute exercise round to aid the assessor in delivering the test. Criteria for termination of the test was maximum HR reached, failure to maintain required step frequency or power and finally volitional exhaustion of the volunteer.

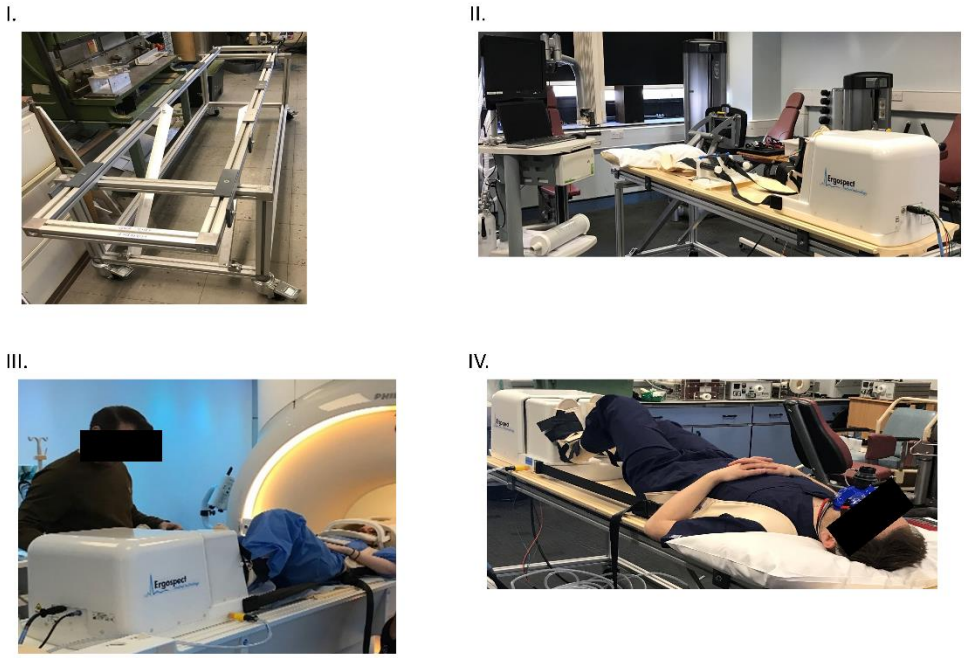



***Figure 3-4.*** *The custom-built MR bed frame for supine exercise testing in the exercise physiology laboratory. II) Philips Achieva MR bed mounted to a custom-built frame with the Ergospect Cardiostepper module secured. (III.) Experimental Ergospect setup for exercise within the Phillips Ingenia scanner (IV.) Experimental Ergospect setup during supine incremental stepping exercise and online gas analysis.*

Results showing the efficacy of using the Cardiostepper module to enable accurate quantitation of supine $VO_2$ peak are provided in Table 3-2 and Figure 3-5.

Supine $VO_2$ and heart rate showed a clear linear response to steady state exercise at incremental workloads whilst minute ventilation and expired $CO_2$ volumes increased linearly during the initial response to incremental steady state exercise, before exhibiting a curvilinear response

toward the later stages of the CPET (Fig. 3-5 to 3-6) due to the excess $CO_2$ produced during the later exercise stages and associated hyperventilation.

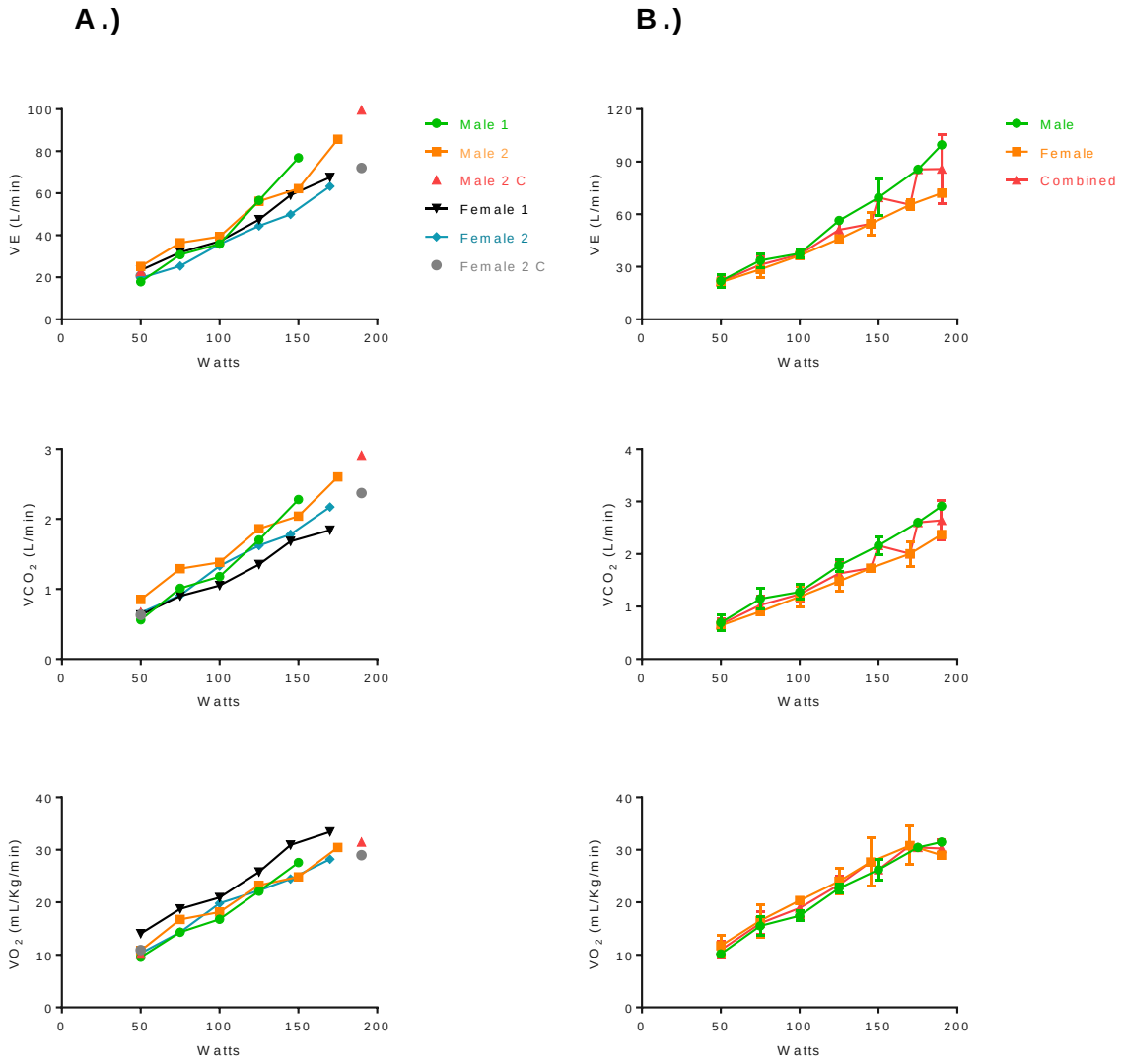



***Figure 3-5.*** *CPET parameters during incremental exercise testing on the 7T compatible Ergospect Cardiostepper in healthy volunteers. A.) Individual subject data with conformation testing. B.) Mean ± SD for male, female and combined group data.*

| Group | VE (L/min) | $VCO_2$ (L/min) | $VO_2$ (ml/kg/min) | Watts | Heart Rate | RPE |
|---|---|---|---|---|---|---|
| All | 79.0 ± 14.3 | 2.4 ± 0.4 | 30.4 ± 2.6 | 175 ± 19 | 155.6 ± 9.5 | 16.7 ± 1.3 |
| Female | 69.8 ± 3.2 | 2.1 ± 0.4 | 31.2 ± 3.1 | 180 ± 14 | 165 | 16 ± 1.41 |
| Male | 88.3 ± 16.1 | 2.6 ± 0.5 | 29.5 ± 2.8 | 170 ± 28 | 151 ± 7.1 | 17.5 ± 0.7 |

***Table 3-2.*** *Peak CPET data displayed for the healthy volunteers, and female and male subgroups.*

The repeatability of the Cardiostepper module was assessed by repeated $VO_2$ peak assessment in the same volunteer. Test-retest variation was confirmed as acceptable, with a supine $VO_2$ peak of 30.6 ± 1.2 ml/kg/min, giving a coefficient of variation of 3.9% (Table 3-3 & 3-4 Fig.3-6).

| | VE (L/min) | $VCO_2$ (L/min) | $VO_2$ (ml/kg/min) | HR | RPE | Watts |
|---|---|---|---|---|---|---|
| M ± SD | 87.3 ± 12.8 | 2.6 ± 0.4 | 30.6 ± 1.2 | 152± 5 | 17 ± 1 | 180.0 ± 8.7 |
| CV (%) | 14.7 | 13.8 | 3.9 | 3.5 | 3.3 | 4.8 |

***Table 3-3.*** *Displaying CPET repeatability data for one healthy volunteer (incremental exercise testing and confirmation test data included).*

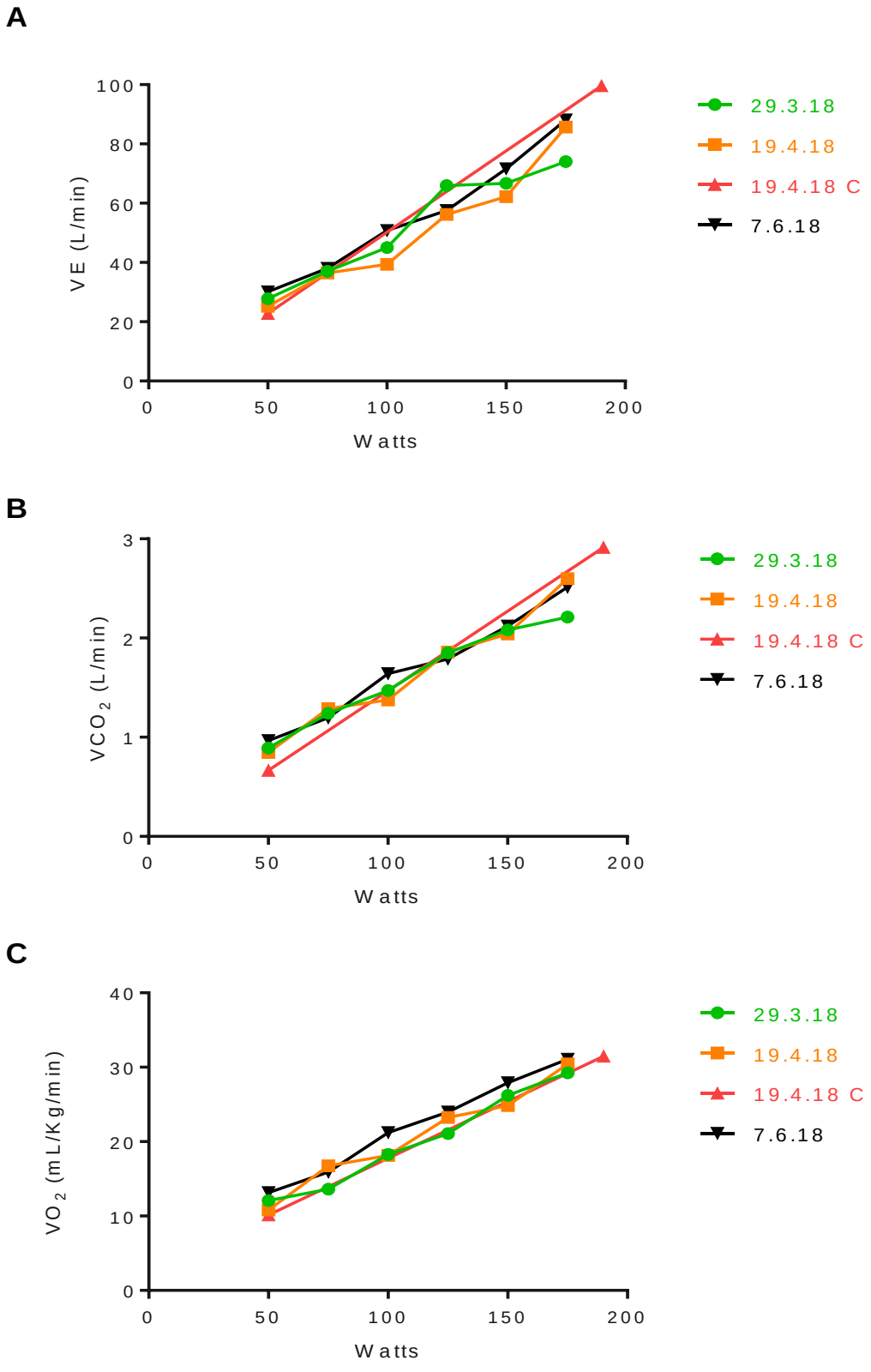



***Figure 3-6****. CPET repeatability testing on one healthy male volunteer on the Ergospect 7T compatible Cardiostepper module. Data shows minute ventilation (VE), expired $CO_2$ ($VCO_2$) oxygen uptake ($VO_2$) heart rate and rate of perceived exertion (RPE) during three separate CPET.*

| Watts | VE (L/min) | CV | $VCO_2$ (L/min) | CV | $VO_2$ (ml/kg/min) | CV | HR | CV | RPE | CV |
|---|---|---|---|---|---|---|---|---|---|---|
| 50 | 26.47 ± 3.15 | 11.9 | 0.84 ± 0.13 | 15.1 | 11.55 ± 1.33 | 11.5 | 96 ± 10 | 11 | 7.25 ± 0.5 | 6.9 |
| 75 | 37.17 ± 0.81 | 2.2 | 1.24 ± 0.05 | 3.8 | 15.44 ± 1.62 | 10.5 | 116 ± 6 | 6 | 9.67 ± 0.58 | 6 |
| 100 | 45.07 ± 5.69 | 12.6 | 1.5 ± 0.13 | 8.9 | 19.22 ± 1.73 | 9 | 127 ± 9 | 7 | 12.67 + 0.58 | 4.6 |
| 125 | 59.9 ± 5.32 | 8.8 | 1.83 ± 0.04 | 2.1 | 22.77 ± 1.52 | 6.7 | 137 ± 9 | 7 | 13.67 ± 1.15 | 8.5 |
| 150 | 66.85 ± 4.75 | 7.1 | 2.08 ± 0.04 | 1.9 | 26.33 ± 1.53 | 5.8 | 144 ± 9 | 7 | 15.33 ± 1.15 | 7.5 |
| 175 | 82.62 ± 7.53 | 9.1 | 2.44 ± 0.2 | 8.3 | 30.26 ± 0.94 | 3.1 | 152±5 | 4 | 17.33 ± 0.58 | 3.3 |

***Table 3-4****. Mean ± SD and coefficient of variation for minute ventilation rate (VE), expired $CO_2$ ($VCO_2$), Oxygen uptake ($VO_2$), heart rate and rate of perceived exertion at each workload across three supine CPET carried out in the same individual on different days.*

Collectively these developmental data confirmed that the Cardiostepper module could be used to exercise healthy volunteers at the same relative workload (50% $VO_2$ peak). These data enabled us to begin patient studies, quantifying the primary MRI endpoints during exercise (cardiac output, cerebral blood flow, regional perfusion, oxygen extraction).

However, upon recruitment of the first healthy volunteer into the main study, a software error was identified during the incremental exercise testing. The dynamics of VE, $VCO_2$ and $VO_2$ from the online gas analysis showed clear issues (Fig.3-7) and subjective feedback from the volunteer indicated no clear transition between prescribed workloads and varying difficulties throughout the exercise test. This was initially attributed to a calibration fault, and a confirmation test was performed following re-calibration of the device, but subsequent analysis of the Ergospect software data revealed severe fluctuations in power throughout the protocol resulting in issues in expired gas variables. Further, there were significant discrepancies in the workload achieved, markedly greater than those we had previously observed during development testing with healthy volunteers (Fig 3-6).

Incremental exercise testing was re-performed on the same male volunteer who performed the initial validation work (Fig. 3-8). This revealed significant reductions in minute ventilation, expired $CO_2$ and oxygen uptake across workloads from 100W onwards (Fig. 3-8). The stark difference in peak workload attained (Fig. 3-8) confirmed a software issue on the device. In order to test this assumption, repeated incremental exercise testing was performed on the male volunteer who performed the initial development experiments where existing CPET data was available for comparison (Table 3-3 & 3-4, Fig. 3-6). This revealed differences in ventilation rate, expired $CO_2$ and $VO_2$ across exercise workloads at 100W and greater (Fig. 3-8). After a period of communication with the Ergospect engineers, it was concluded this necessitated a change of the complete 7T compatible Ergospect system and associated control boxes to a new model of a 3T compatible Ergospect system. This was delivered to the University of Nottingham in October 2018.

| **Test Date** | **VE (L/min)** | **$VCO_2$ (L/min)** | **$VO_2$ (ml/kg/min)** | **HR** | **RPE** | **Watts** |
|---|---|---|---|---|---|---|
| M ± SD | 92.46 ± 14.74 | 2.61 ± 0.31 | 31.03 ± 1.29 | 154.19 ± 6.09 | 17.50 ± 0.58 | 203.75 ± 48.02 |
| CV (%) | 15.94 | 12.04 | 4.16 | 3.95 | 3.30 | 23.57 |

***Table 3-5.*** *Displaying CPET repeatability data in one healthy volunteer and additional data from a fourth test performed after identification of hardware / software error. Data are presented as M ± SD and includes confirmation test.*

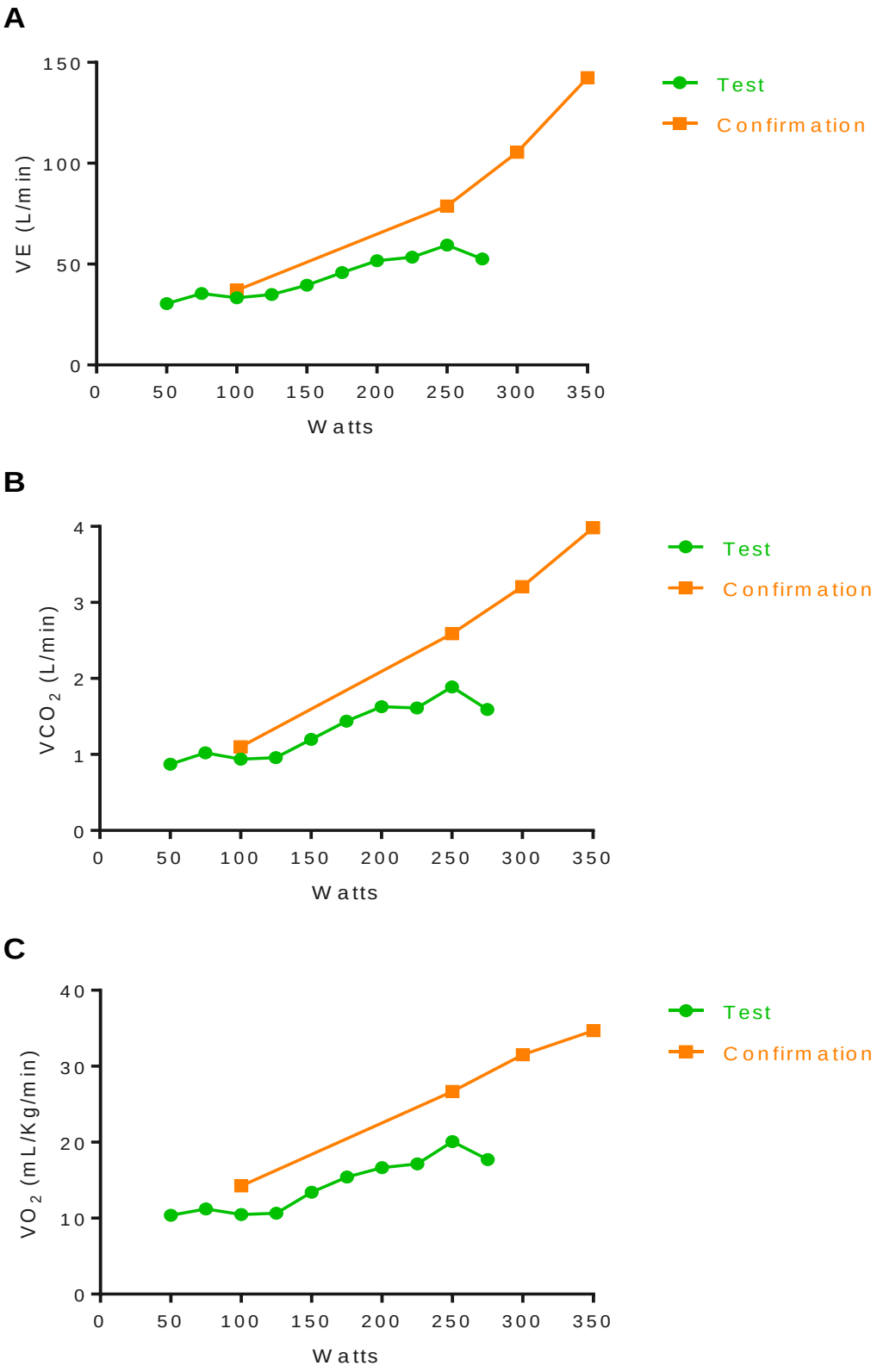



***Figure 3-7****. CPET data showing minute ventilation (VE), expired $CO_2$, oxygen uptake, heart rate and rate of perceived exertion responses to supine incremental exercise. Data shown are individual data points from the CPET and confirmation test from the first healthy volunteer.*

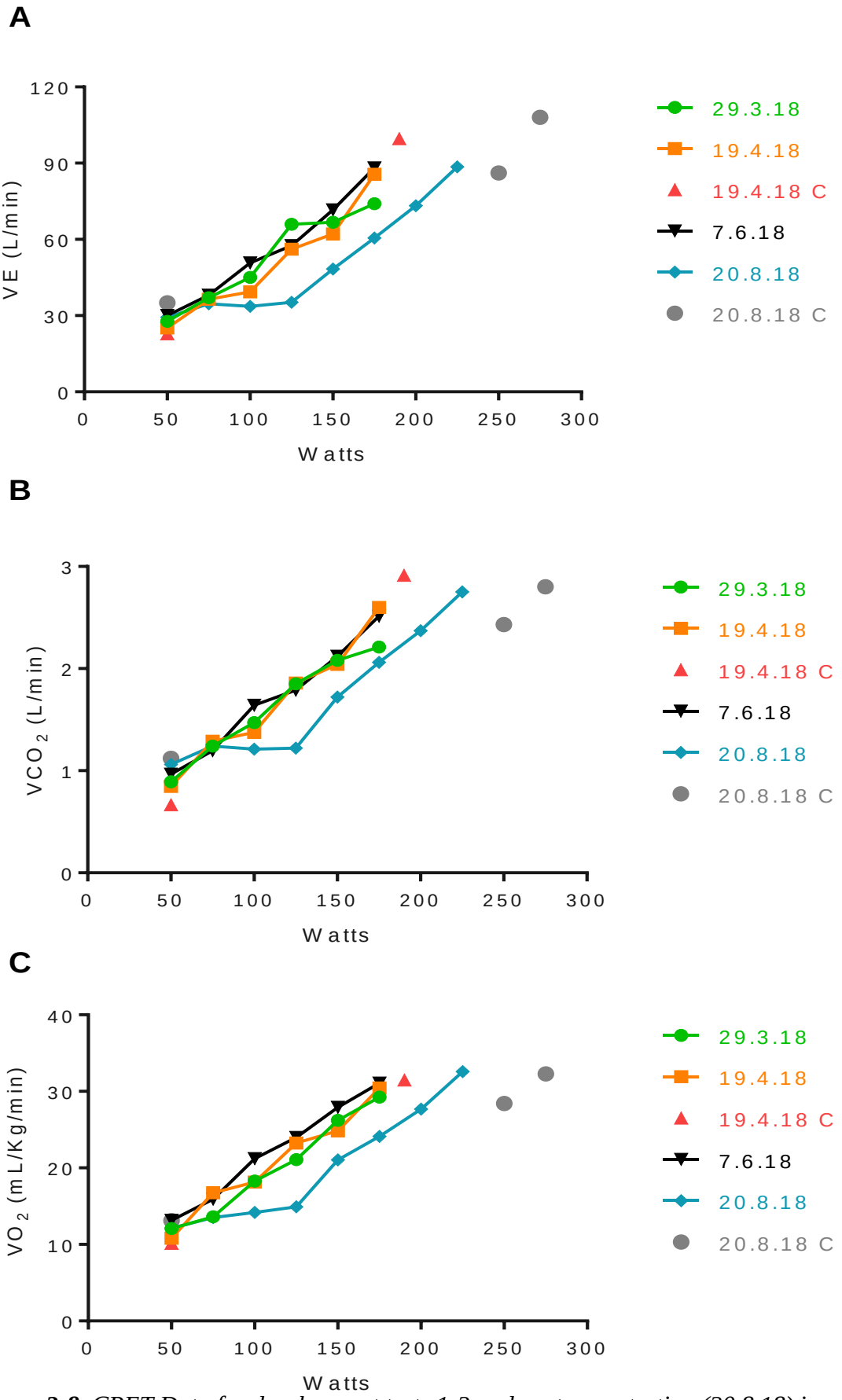



***Figure 3-8****. CPET Data for development tests 1-3 and post error testing (20.8.18) in male HV.*

### 3.1.2 Evaluating the 3T Compatible Ergospect Cardiostepper

The experiments described in Section 3.1.1 were then repeated in order to establish the efficacy of the 3T compatible Ergospect Cardiostepper module to ascertain supine $VO_2$ peak. Results are shown in Table 3-6-9 and Fig 3-9 & 10, which highlight the capability of the 3T compatible Cardiostepper module to reliably ascertain supine $VO_2$ peak during repeated CPET in the same volunteer.

| Experiment | **Age** | **Height** | **Weight** | **BMI** |
|---|---|---|---|---|
| Development | 28 ± 2 | 169 ± 9 | 71 ± 11 | 25 ± 2 |
| Repeatability | 26 | 173 | 80 | 27 |

***Table 3-6.*** *Demographic of healthy volunteer group (n= 3) who performed validation of the Ergospect 3T compatible Cardiostepper module.*

| **VE (L/min)** | **$VCO_2$ (L/min)** | **$VO_2$ (mL/kg/min)** | **Heart Rate** | **Watts** | **RPE** |
|---|---|---|---|---|---|
| 75.6 ± 10.0 | 2.50± 0.3 | 31.0 ± 4.6 | 157 ± 14.0 | 225 ± 25 | 18.3 ± 1.2 |

***Table 3-7****. Peak CPET data from validation of Ergospect 3T Cardiostepper module.*

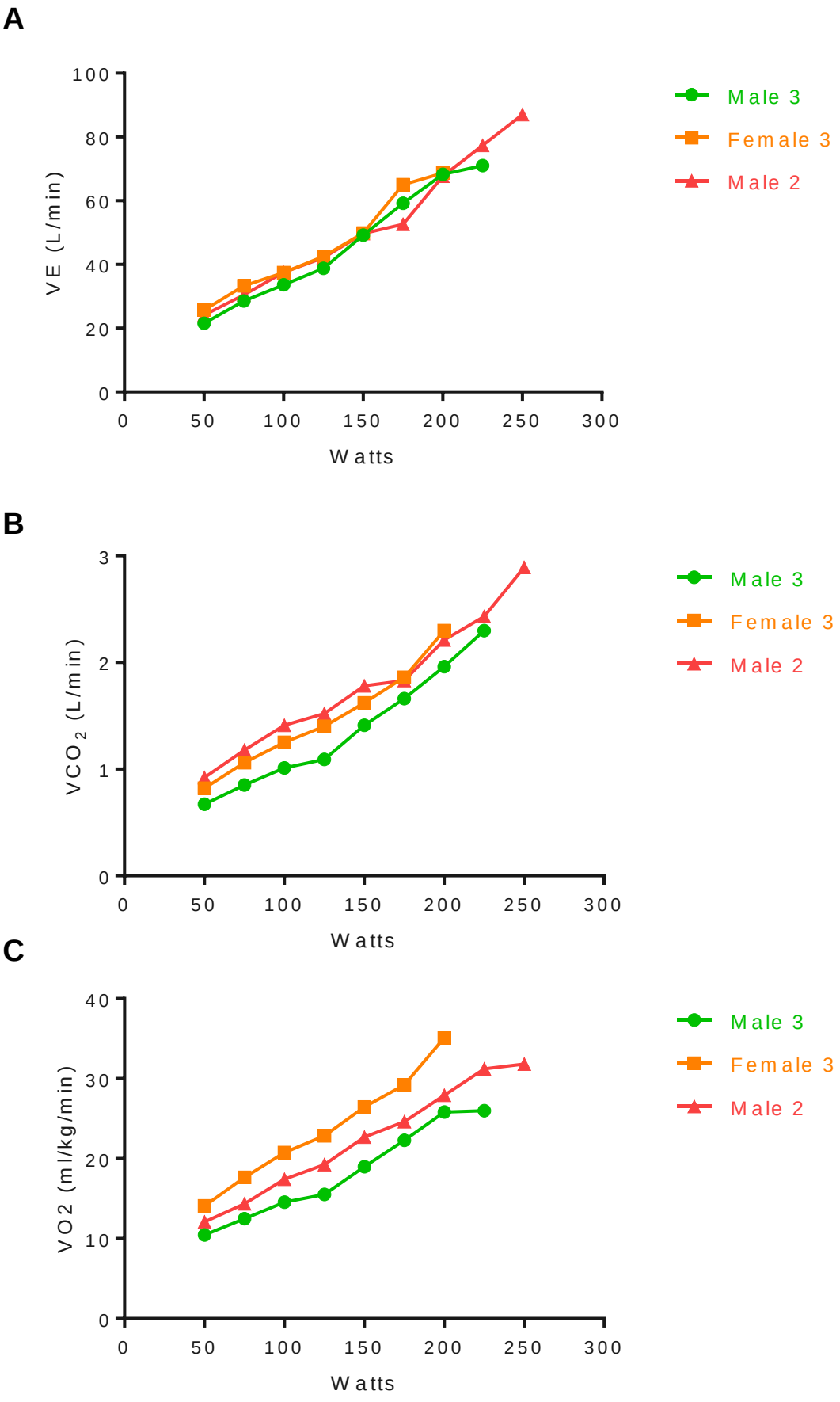



***Figure 3-9.*** *CPET data from three healthy volunteers using the 3T compatible Ergospect Cardiostepper module.*

| | **VE (L/min)** | **$VCO_2$ (L/min)** | **$VO_2$ (ml/min/kg)** | **HR** | **RPE** | **Watts** |
|---|---|---|---|---|---|---|
| **N=5 CPETs (12-weeks)** | 91.64 ± 13.25 | 2.66 ± 0.17 | 30.81 ± 1.81 | 154.43 ± 8.91 | 18.00 ± 1 | 235.00 ± 13.69 |
| **CV** | 14.5 | 6.3 | 5.9 | 5.8 | 5.6 | 5.8 |
| **N=3 CPET's (3-weeks)** | 92.11 ± 17.84 | 2.67 ± 0.23 | 31.56 ± 0.3 | 154 ± 12 | 18.67 ± 0.58 | 233 ± 14 |
| **CV** | 19 | 9 | 1 | 8.0 | 3.1 | 6.2 |

***Table 3-8.*** *CPET data from a healthy male volunteer during five CPET performed over 12-weeks and during three of the later CPET tests, performed over a three-week period. Data presented as M ± SD and CV.*

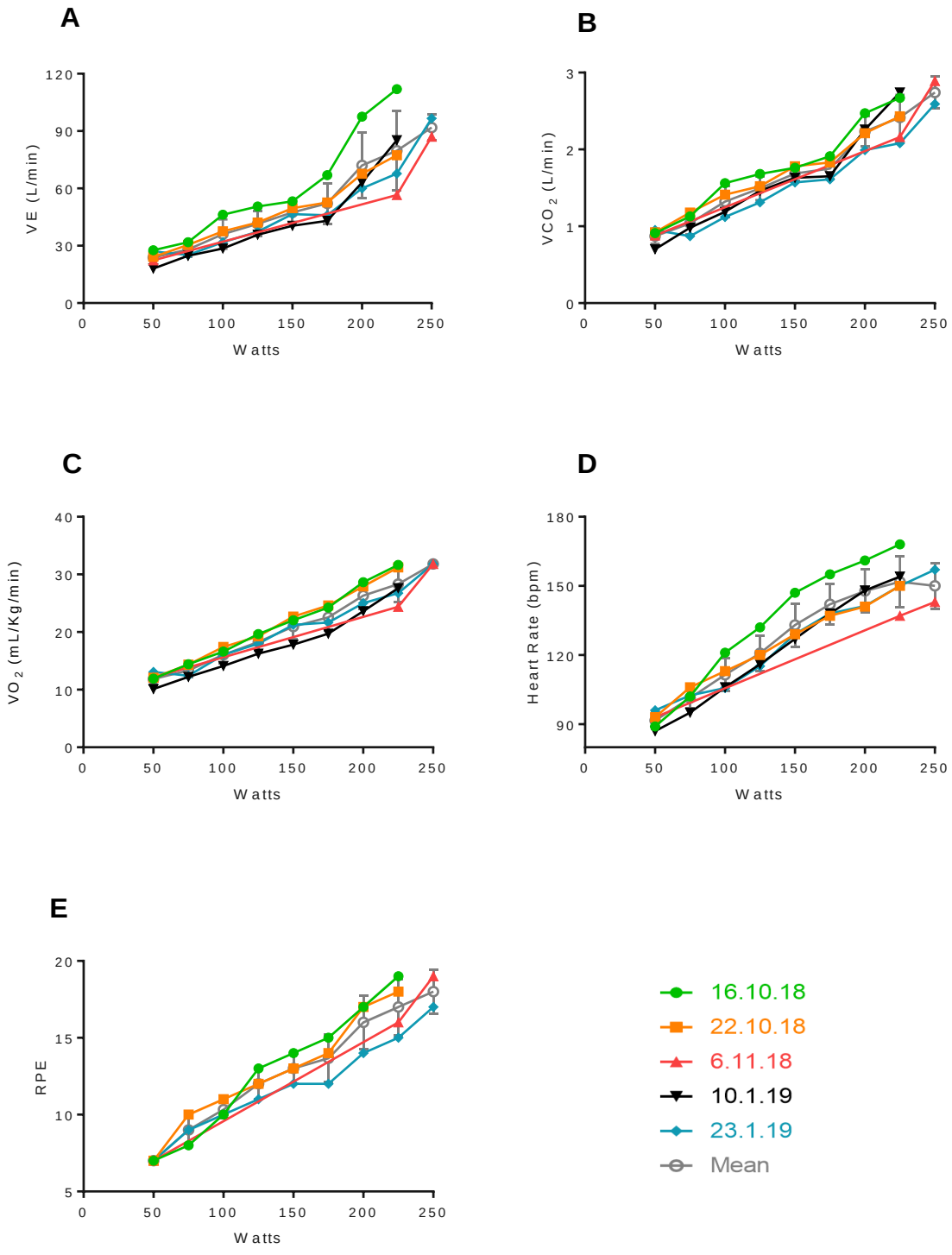



***Figure 3-10**. CPET repeatability data from five CPET in a single healthy volunteer using the 3T compatible Ergospect Cardiostepper system over a 12-week development period*

These data confirmed the efficacy of the replacement 3T-compatible Cardiostepper module to achieve accurate quantitation of supine $VO_2$ peak. Both minute ventilation and expired $CO_2$ volumes display curvilinear responses to exhaustive incremental exercise, whilst heart rate and oxygen uptake demonstrate a clear linear response to increasing exercise intensity. These findings were repeatable, with supine $VO_2$ peak demonstrating a coefficient variation of 6% (Table 3-8) across multiple CPET over a 12-week period and 1% (Table 3-8) over a three-week measurement period. Following this initial 18-month developmental period, these data now enabled within-bore exercise experiments to be undertaken.

| **VE (L/min)** | **$VCO_2$ (L/min)** | **$VO_2$ (ml/kg/min)** |
|---|---|---|
| 75.6 ± 5.8 | 2.5 ± 0.2 | 30.9 ± 2.7 |

***Table 3-9.*** *Minute ventilation, expired $CO_2$ and oxygen uptake ($VO_2$) volumes for Ergospect 3T Cardiostepper (N=3) in two separate cohorts of healthy young volunteers. Data presented as M ± SD.*

### 3.1.3 MRI Protocol Development to study central physiology with the Cardiostepper

The MRI exam card for the assessment of cardiac output and cerebral blood flow, was developed in parallel with the troubleshooting investigations to resolve the Cardiostepper issues described in Sections 3.1.1 and 3.1.2. An initial series of experiments was performed to assess the feasibility of performing supine stepping using the Ergospect 3T compatible Cardiostepper whilst collecting data within the 3T Ingenia scanner. Initial experiments checked the feasibility of obtaining the cardiac MRI measures and brain PC-MRI scans using the head & neck RF coil for receiving the signal (shown in Figure 3-1) together with the posterior receive RF coils built into the scanner bed. This had the advantage of allowing ease of performing

exercise, as compared to the anterior body coil, which is placed over the subject for resting cardiac scanning, but this would have been limiting for performing supine exercise. The 3T compatible Cardiostepper module was mounted to the 3T Ingenia scanner bed using a vacuum pump operated remotely in the control room. The control modules and external PC connections were set-up in order to ascertain an estimation of pre-scanning set up times (see Figure 3-4 for schematic of full kit set up). A healthy volunteer was set up on the MR scanner bed with their feet strapped into the Cardiostepper module and their knee angle secured at an angle of 25-30˚ at the bottom of the pedal stroke.

Patient motion during MRI scanning is a significant challenge. Motion results in image artefacts, blurring and the need to discard data which cannot be adequately motion corrected. Within-bore exercise protocols exacerbate this issue, and previous studies from our group using a supine-cycle ergometer restricted exercise intensity to ≤50% $VO_2$ peak to avoid excessive head motion identified at higher intensities. In light of this, we carried out pilot experiments to assess head motion during supine stepping exercise on the Ergospect 3T Cardiostepper module. Two healthy volunteers who completed a supine CPET at the DGHPU were then scanned on the Phillips 3T Ingenia. Volunteers were secured into the Cardiostepper ensuring an identical position to that used during the CPET in the physiology laboratory. Consistent positioning of the volunteers was confirmed by measurement the length of the straps connecting the waistcoat of the patient and by measurement of the knee angle by an MRI compatible goniometer.

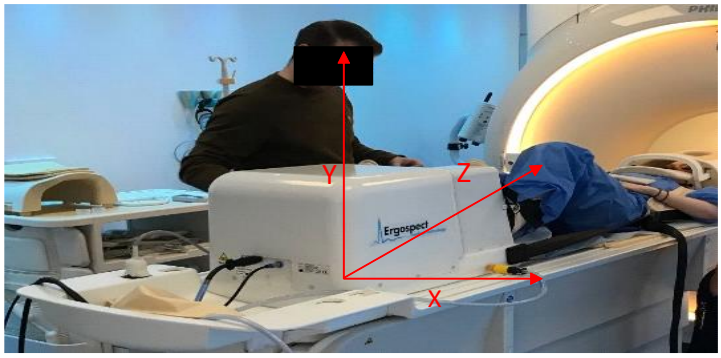
Following baseline scanning, the volunteers were instructed to step to the beat of a metronome played at 70 beats per minute. Following three minutes of continuous stepping exercise to allow the volunteer to reach steady state, scans were acquired at four submaximal exercise intensities based upon the volunteers predetermined supine $VO_2$ peak. In the first volunteer, no instructions were given pertaining to motion during exercise within the scanner and no head padding was added to the head coil (Fig 3-11, B). However, the second volunteer was briefed

on the importance of preventing excessive head motion and padding was added to the head coil in an attempt to fix the head into a secure and comfortable position during image acquisition (Fig 3-11, C).

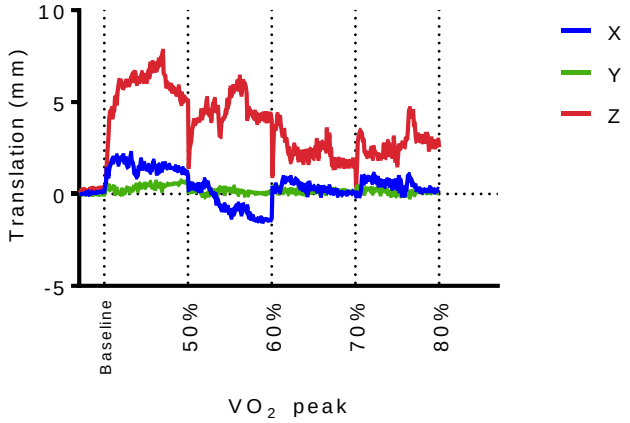
| Experiment | Age | BMI |
|---|---|---|
| Motion & Cardiac (N=2) | 40 ± 1 | 24 ± 2 |
| CBF (N=3) | 30 ± 9 | 23 ± 2 |

***Table 3-10**. Demographic data for healthy volunteers in MRI protocol development.*

**A .)**




**B .)**




**C .)**

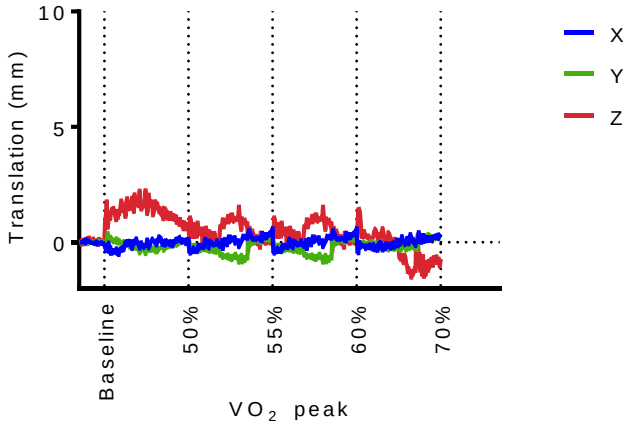



***Figure 3-11.*** *Experimental MRI exercise set up on the Philips Ingenia with schematic representation of the X, Y, Z planes. B.) Volunteer head motion in the X, Y, and Z plane during supine stepping exercise without head padding and C.) with head padding added to the head and neck coil.*

ASL scans label and control images were analysed using FSL to generate motion plots for each individual exercise bout. Data were then plotted to visualise translational head motion (X, Y and Z planes) during supine stepping exercise for each subject (Fig. 3-11).

These data demonstrate a clear reduction in the magnitude of head motion when the volunteer was briefed on the importance of remaining still and received additional padding to the head coil (Fig 3-11). At 50% $VO_2$ peak, where the greatest magnitude of head motion was observed in both volunteers, head padding and verbal briefing reduced peak head motion (translation through the Z plane, mm) by 77% relative to the non-padded condition (M ± SD, 1.34 ± 2.25 vs 5.78 ± 7.78 mm). Despite a higher magnitude of head motion for the non-padded relative to the padded condition, head motion was not dependent on exercise intensity (Fig. 3-11, B & C). Both data sets demonstrate that the highest level of relative motion occurred within the first exercise bout. This suggests that familiarisation prior to the initial exercise workload may be a more important consideration for reducing head motion than relative exercise intensity during supine stepping exercise. To circumvent this issue, during the full experimental protocol, volunteers exercised for 3-minutes at 50% $VO_2$ peak prior to any data acquisition. This served as an additional familiarisation phase to minimise the risk of excessive head motion. Further, this ensured that measurements of central haemodynamics and oxygen metabolism were performed with the volunteer in steady-state.

In addition, in this pilot session, we were able to successfully quantify real-time cardiac output under resting conditions and during supine exercise at 50% supine $VO_2$ peak (Fig. 3-12 A & B) in both healthy volunteers using PC-MRI. Resting state cerebral blood flow values were also calculated by 2D Flow scans acquired during developmental testing. Global cerebral blood flow was calculated as the sum of velocity and vessel area across the internal carotid and basilar arteries (Fig 3-12, C).

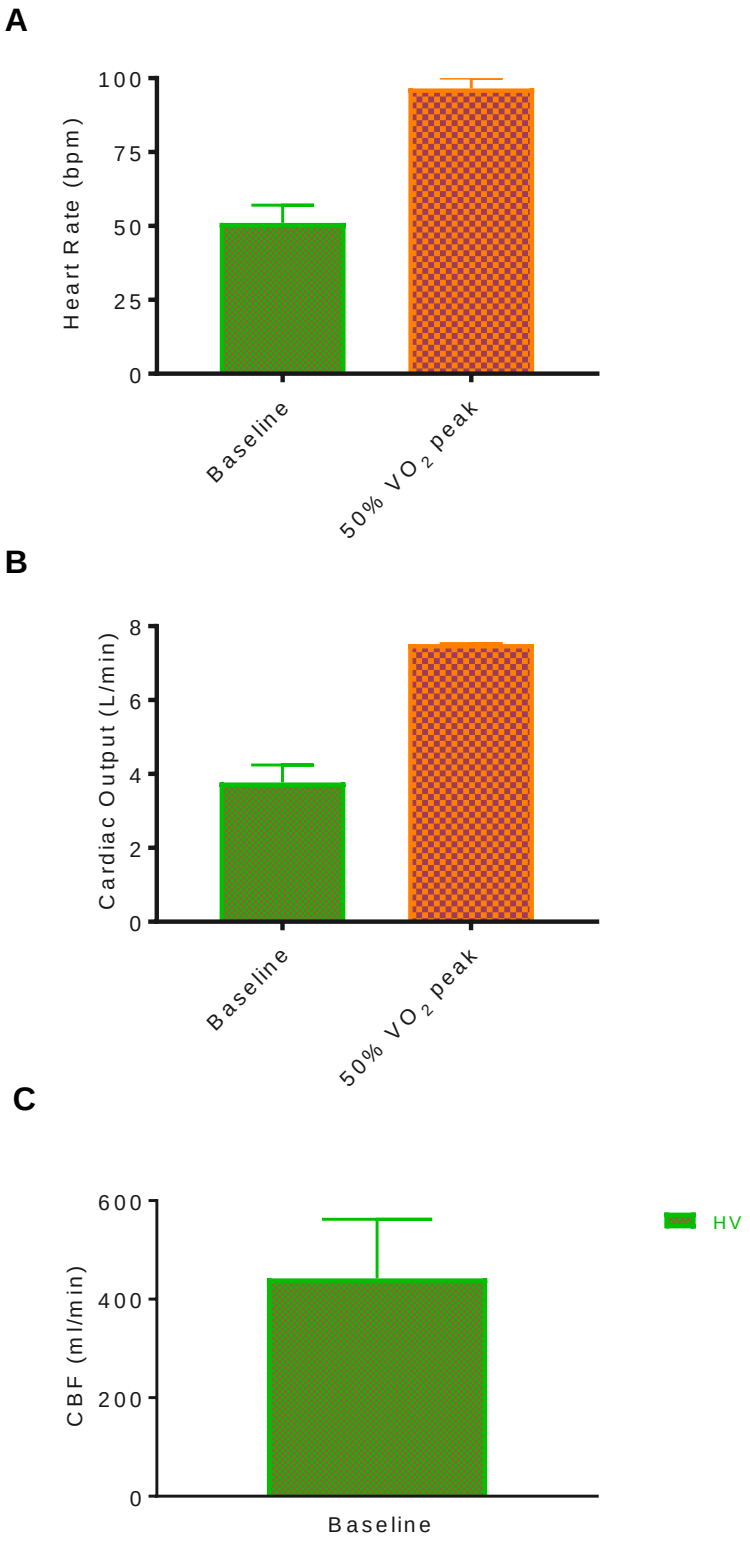



***Figure 3-12.*** *A.) Heart rate B.) Cardiac output during rest and supine exercise at 50% $VO_2$ peak. C.) Baseline global cerebral blood flow, in three healthy volunteers.*

### 3.2 Assessing peripheral deconditioning using the Trispect and $^{31}$P MRS

#### 3.2.1 Assessment of the Trispect and its influence on $^{31}$P MR spectra

Using the 3T compatible control boxes and Trispect module, tests were performed to assess the quality of the $^{31}$P MRS measures. This was particularly important as the Trispect module is positioned within the magnet bore during measurements, and thus could introduce RF noise if not correctly uncoupled, unlike the Cardiostepper module that is positioned outside the magnet bore (see Figure 3-3). Figure 3-13 shows the experimental set-up of the Trispect prior to movement of the volunteer such that the Trispect is then positioned within the centre of the magnet bore. During the initial experimental test, significant RF noise was seen on the Survey scans of the human calf (Fig. 3-14 I & II) and the $^{31}$P MRS of the medial gastrocnemius (Fig. 3-14 III & IV). Through a series of repeated scans, it became apparent that the new 3T compatible Ergospect control boxes were porting RF noise into the 3T Achieva scanner.


***Figure 3-13**. Experimental Trispect set-up for $^{31}$P MRS experiments on the Philips Achieva.*

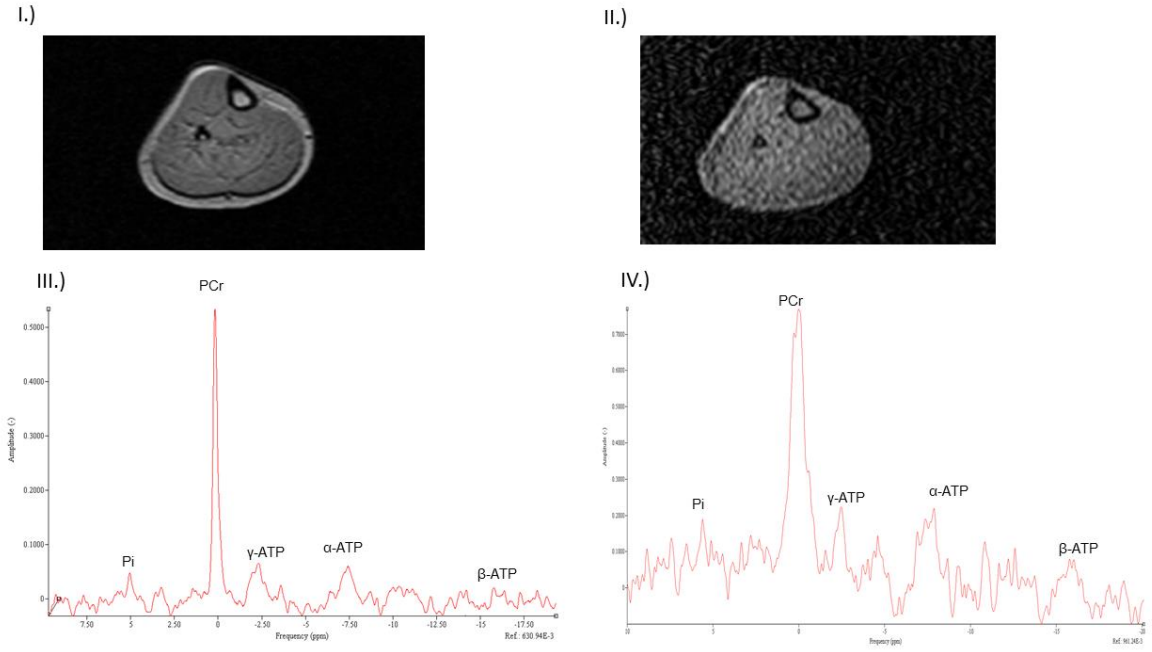



***Figure 3-14****. Axial calf images and localised $^{31}P$ MRS of medial gastrocnemius muscle with the power supply to the Ergospect interface box disconnected OFF (I & III.) and ON (II & IV.) Note the increase in noise with the interface box turned ON.*

A series of experiments were then performed to investigate the source of the RF noise issue. Firstly, benchtop tests outside the MR scanner were performed using a spectrum analyser. Using a loop search coil, it was discovered that it was possible to observe a raised level of electromagnetic interference (EMI) emanating from the Ergospect interface box through the external cable (either with or without the control box connected and operating), Fig 3-15 I & II. Upon opening the interface box, a search coil revealed evidence of wideband and harmonic noise at 50 – 150 MHz region emanating from the fibre receiver and sensor interface modules.

Note the Larmor frequency of $^{1}H$ and $^{31}P$ at 3T is 128 and 52 MHz respectively.

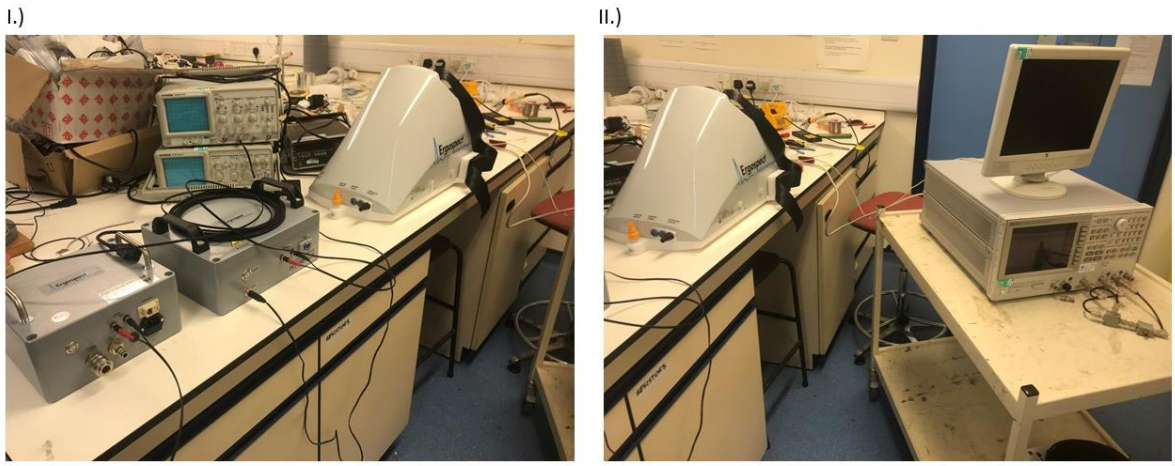



***Figure 3-15****. Bench top experiments using a network analyser to assess RF noise from the Ergospect system prior to MRI experiments. Ergospect control and interface boxes and Trispect device (I) connected for bench top testing with the network analyser (II).*

The Trispect module was then set-up in the 3T Achieva for further testing, here the effect of the position of the Trispect module on the RF noise was assessed as illustrated in Figure 3-16.

In Position B, the location where the Trispect module was within the magnet bore and so within the Q-Body Coil (QBC), there was significant wideband noise present on the survey scan as shown on the phantom and *in-vivo* (Figure 3-16, II-VI). Removing the Trispect module from the bore to Position A outside of the Q-body coil (the position the Trispect module would be if a head was being scanned instead) led to reduced noise. With the Trispect module at Position B within the bore, the noise could also be suppressed by either switching off the interface (IF) box, or disconnecting the Trispect. This demonstrated that the source of the RF noise was not the cable pick-up of EMF.

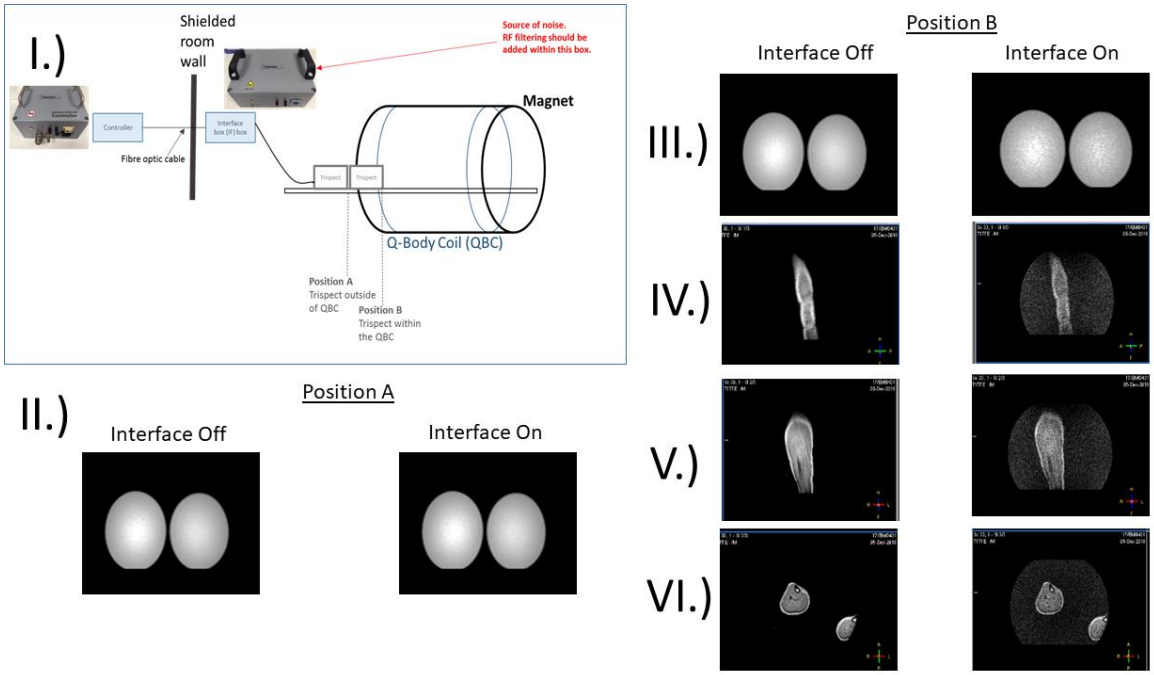



***Figure 3-16.*** *Schematic of assessing position of Ergospect Trispect system on the Phillips 3T Achieva, and associated $^{1}H$ MRI data in positions A & B (I.) from phantom (II & III.) and human calf scans in the sagittal, coronal and axial planes (IV-VI) in positions A & B. Images show a clear increase in RF noise in position B compared to position A for both the phantom and in-vivo scans.*

In summary, these bench-top and MRI experiments collectively demonstrated that the MR compatible Ergospect interface box operated via connection to a control box placed in the console room using a fibre optic cable connection placed through a waveguide, was generating significant wideband and harmonic noise in the 50 – 150 MHz range. This resulted in significant noise in both $^{1}H$ images and $^{31}P$ spectra when the Trispect module was in position B.

In discussion with Dr Paul Glover at SPMIC, the interface box was inspected further. It was clear that no attempt at electromagnetic interference (EMI) filtering had been applied to the MR compatible interface box. A low-pass filter was constructed and installed by Dr Glover, and a discussion held with Ergospect to recommend that RF filters should be added to the circuitry within the Interface box. Ergospect engineers dispatched a replacement interface box with additional ferrite shielding in January 2019.

### 3.2.2 Test re-run using the new interface box.

Despite the new interface box with ferrite filtering provided by Ergospect reducing the level of RF noise at 128 MHz for $^{1}H$ imaging, an increase in RF noise was still apparent (Fig 3- 17-19). Further, there was a clear >10-fold increase in the noise floor for $^{31}P$ measures with the Interface box turned on during phantom scanning, indicating broadband noise (Fig. 3-18). A series of acquisitions using a localised $^{31}P$ sequence of the medial gastrocnemius revealed that the ferrite shielding did not completely suppress the delivery of RF noise into the bore of the magnet. Figure 3-19. I.) shows a $^{31}P$ spectra collected with the newly delivered interface box with additional ferrite shielding turned ON. Keeping the control box & interface turned ON, the Trispect module was moved as close as possible to the Q-body coil, which decreased the SNR further (Fig. 3-19 (II). The interface box was then moved to the console room, but remained connected to the Trispect device via the sensor cable placed through the waveguide. Again, poor SNR was evident (Fig. 3-19, III). This was improved by turning off the power supply to the interface box (Fig 3-19. IV). Next, we maintained the power supply to the interface box but disconnected the sensor cable from the Trispect (Fig. 3-19 V.), this improved the SNR, which was consistent with a baseline $^{31}P$ spectra was collected without the Ergospect equipment present (Fig 3-19, VI). These data reaffirmed that the power supply from the interface box, which connects directly to the Trispect module via the sensor cable, was responsible for porting RF noise into the bore of the magnet. All conditions where a connection was formed between the interface box and Trispect module showed significant RF interference (Fig 3-19 (I-III), whereas switching the interface off (Fig 3-19. (IV) or disconnecting the sensor cable (Fig 3-19. (V) produced consistent $^{31}P$ spectra to that observed without the equipment present (Fig 3-19. (VI). These data confirmed that the additional ferrite shielding installed by Ergospect had been unsuccessful, since significant RF noise was evident on both $^{1}H$ images (Fig. 3-17-18) and localised $^{31}P$ MRS acquisitions (Fig. 3-18-19).

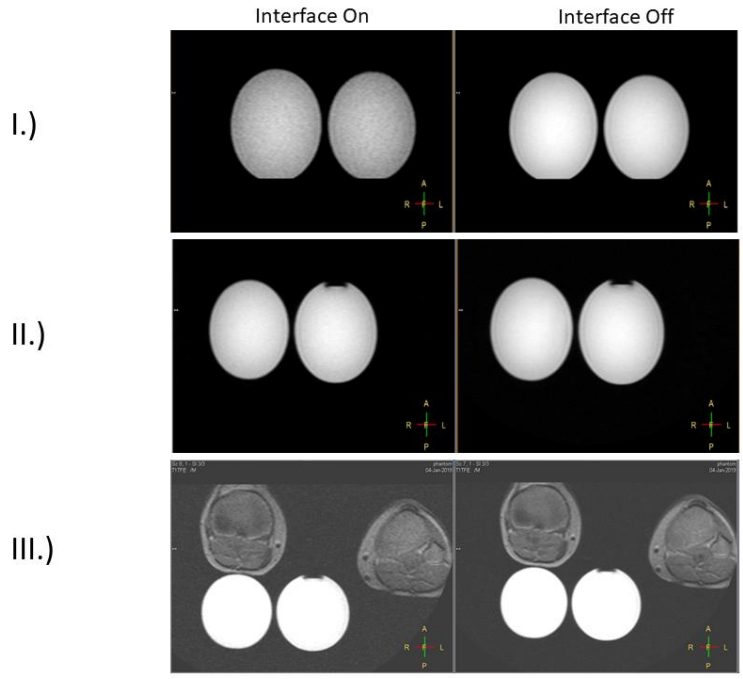
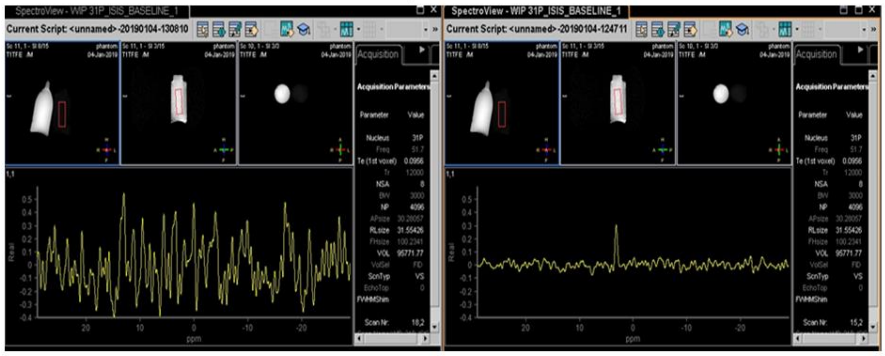



***Figure 3-17.*** *Phantom scans with Trispect within QBC (Position B). (I.) Original 3T compatible interface box December 2018. (II.) New interface box with ferrite filter added January 2019 (III.) Lower limb and phantom scans with new interface box January 2019.*

I.)

II.)



III.)

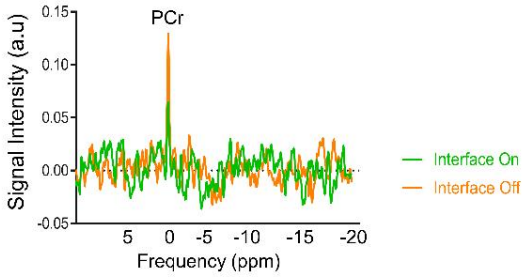



***Figure 3-18***. *$^{31}$P (~ 24 MHz at 3T) spectrum of the phantom scanned with Trispect within QBC (Position B) with I) Interface box turned ON II) Interface box turned OFF – data collected with new box with ferrite filter in box in January. Localised $^{31}$P MRS spectra of the medial gastrocnemius muscle in a healthy volunteer with the new interface box from Ergospect switched OFF and ON (III).*

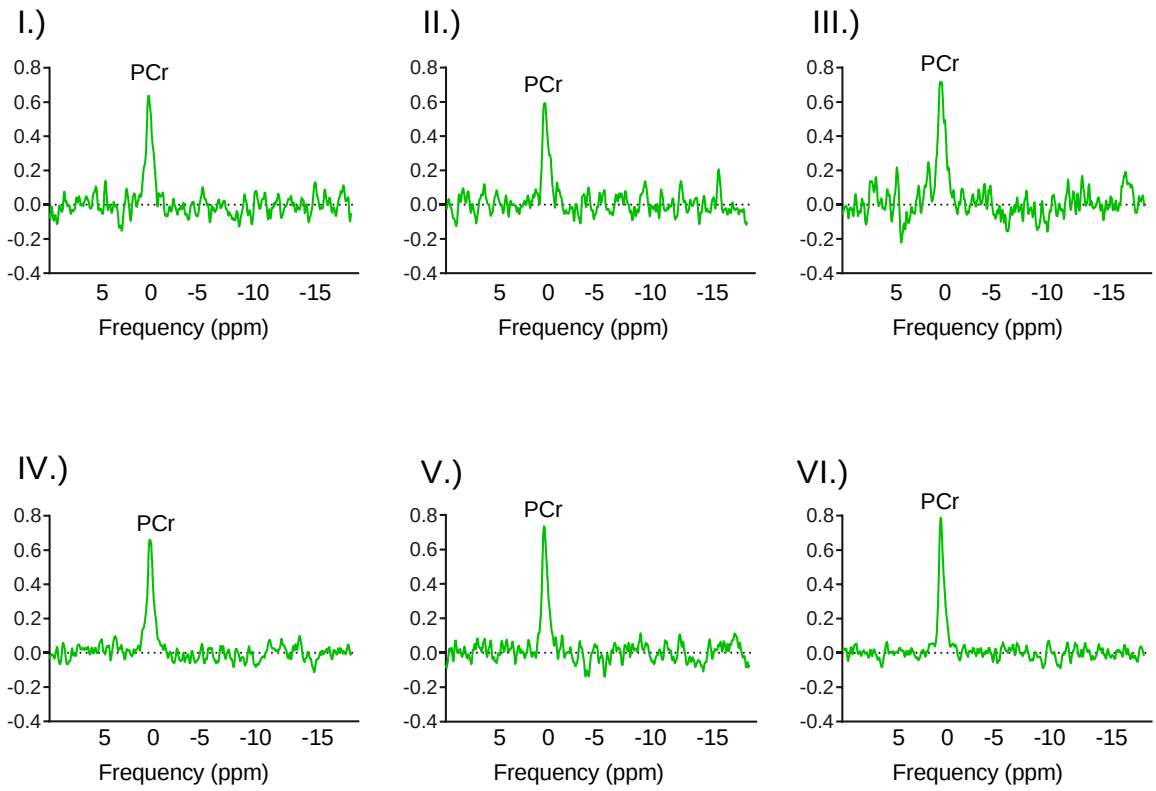



***Figure 3-19**. Series of $^{31}$P MRS spectra collected under different conditions to delineate the mechanism porting RF noise into the scanner. I) New 3T interface box ON. II) Interface box turned ON, Trispect moved close to QB coil. III) Interface turned ON but placed externally at console IV.) Interface turned OFF in the console room V) Interface ON, sensor cable disconnected from Trispect. VI.) No Ergospect equipment present.*

Following further discussion with Ergospect after this finding, it was agreed that they would provide the necessary circuit diagram information for the interface unit. This was to enable completion of the required filter circuitry modifications in house by Dr Glover, with the ferrite installed by Ergospect left in the circuit (Fig. 3-20). Localised $^{31}$P MRS of the medial gastrocnemius performed having added the filter circuitry were consistent when acquired with and without power to the newly modified interface box inside the scan room (Fig 3-21). This confirmed the effectiveness of the additional filter circuitry modifications at preventing the

delivery of RF noise into the bore of the magnet from the interface box to the Trispect device via the sensor cable. Following this evidence of improvement, Ergospect subsequently used this circuitry in their future models of the interface box.

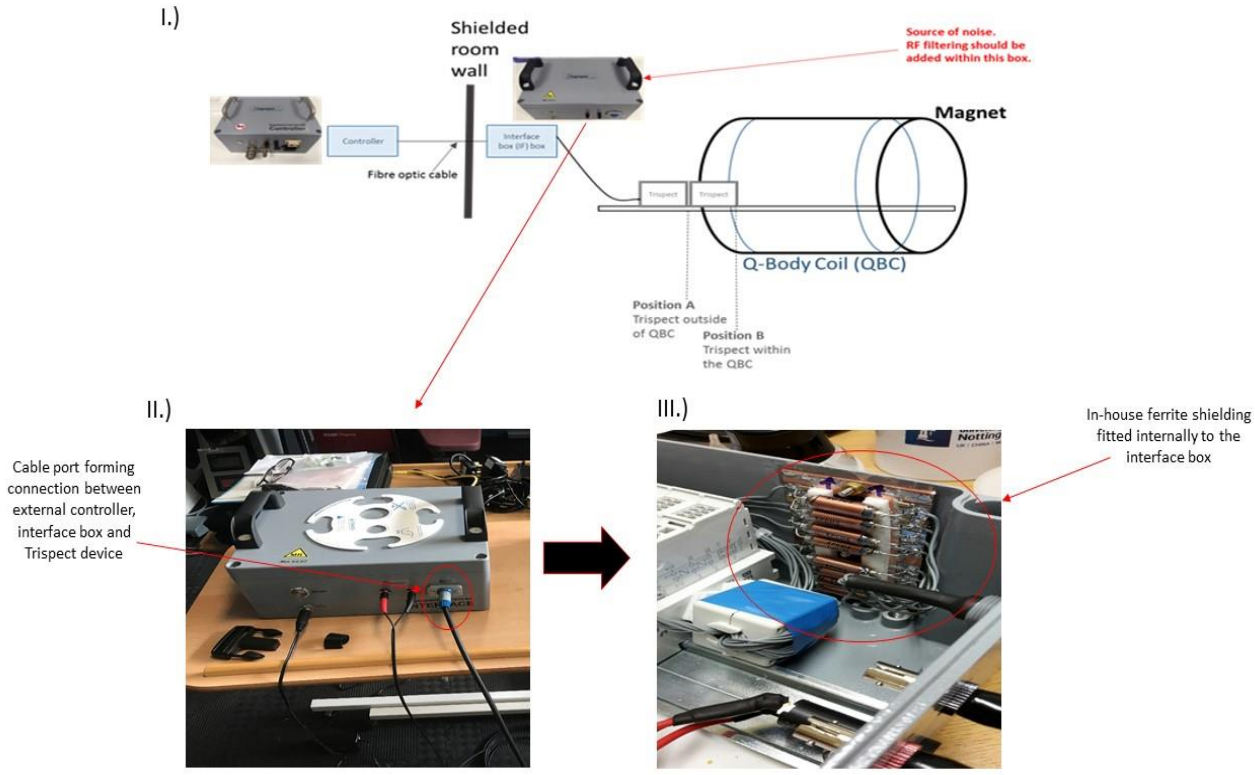



***Figure 3-20****. I.) Overview of experimental Trispect set up. II.) Interface box on benchtop with sensor cable inserted. III.) Internal image of interface box displaying in-house ferrite shielding produced and installation. Note that the shielding is installed internally at the rear of the sensor cable input visible in (II.)*

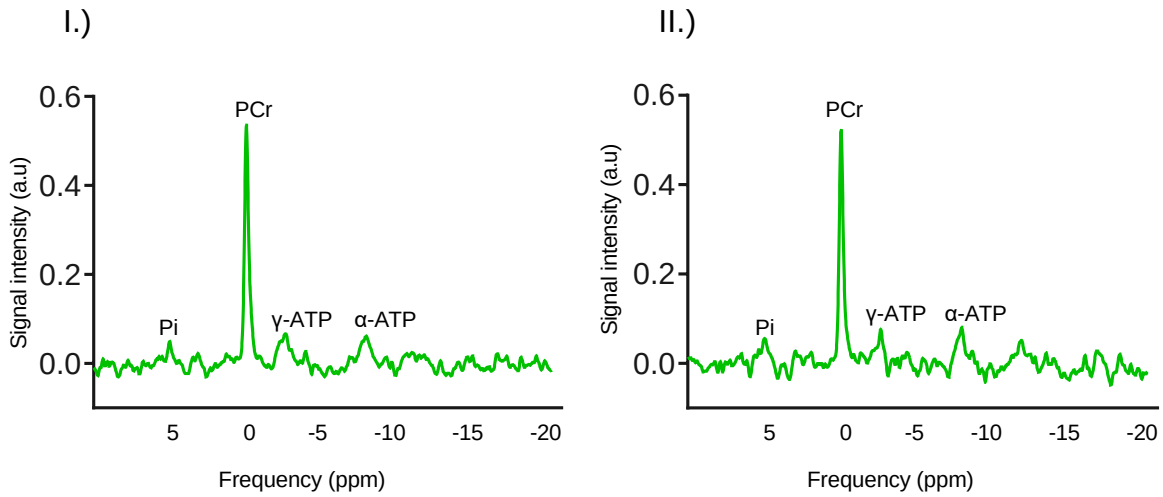



***Figure 3- 21***. *$^{31}$P ISIS acquisition of medial gastrocnemius in a healthy volunteer with newly modified interface box turned OFF (I) and turned ON (II).*

During the course of this work, an additional Ergospect kit was purchased in 2021. Repeat tests were then performed to check that no RF noise issue was apparent in this new equipment, which included the filter circuitry proposed by Dr Glover.

The coefficent of variation in PCr signal between $^{31}$P MRS spectra acquired using the original, problematic interface boxed switched on and off was 23.7%. In contrast, the coefficient of variation using the newly supplied Ergospect interface switched on and off was just 5.3%, demonstrating the effectiness of the hardware updates. Figure 3-22(I) displays the significant reduction in signal-to-noise ratio with the interface switched on relative to off during the intial identification of insufficient RF shielding in January 2019. Figure 3-22(II) shows a comparable signal-to-noise ratio of $^{31}$P MRS spectra using the newly updated 2021 Ergospect interface box. Collectively these data demonstrated that the 2021 equipment was suitable for use during within-bore exercise experiments and could be utilized interchangeably with the original system.

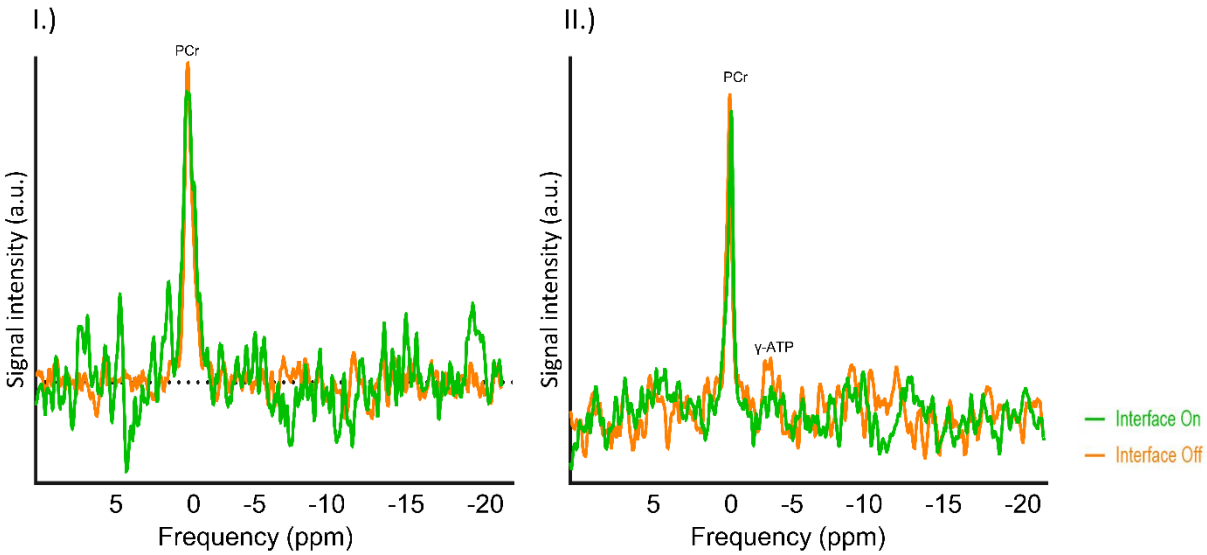



***Figure 3-22**. Example of RF noise interference with interface box switched ON relative to OFF in January 2019 prior to in-house filtering modifications. (II.) Comparison of localised $^{31}P$ ISIS spectra or human calf muscle with additionally supplied interface box turned ON and OFF, showing suppression of RF noise with Ergospect's new filtering system following Nottingham developmental work.*

| **Interface box On / Off comparison** | **PCr signal amplitude (a.u.)** | **CV %** |
|---|---|---|
| January 2019 (Pre-in-house filtering) | 0.052 ± 0.012 | 23.7 |
| April 2021 (Ergospect new filtering) | 0.008 ± 0.0004 | 5.3 |

***Table 3-11**. Comparison of PCr signal intensity during January 2019 RF noise testing of our in-house filtering modifications and later in April 2021 with Ergospect's interface box manufacturer with updated filtering as proposed by Nottingham.*

### 3.2.2 $^{31}$P MRS and $^{1}$H MRI protocol development to study peripheral deconditioning with the Trispect

Two healthy volunteers were scanned to develop the $^{31}$P MRS protocol. $^{31}$P MRS data were collected using a 14 cm $^{31}$P RF coil secured over the medial gastrocnemius. Subjects lay in a

supine position on the MR bed with their dominant limb secured into an MR-compatible plantar flexion ergometer (Trispect, Ergospect). A foam roller was placed under the knee to provide support and to stabilise the limb during exercise. The knee joint was fixed at approximately 30$^{o}$. An inflatable blood pressure cuff was then placed around the leg at the distal femur region. The blood pressure cuff was connected to a cuff inflation device via a hose placed through the waveguide. This device was connected to a portable air compressor at the console which contained an internal air pressure regulator. This allowed remote occlusion of limb blood flow by inflating the blood pressure cuff (Fig 3-13).

$^{1}$H DIXON scans were acquired to image the calf. A series of $^{31}$P Image selected in-vivo spectroscopy (ISIS) scans were then performed under resting conditions and during ischemic plantar flexion exercise, as illustrated in Figure 3-23. Exercise was performed to volitional exhaustion at a standardised workload of 10 Watts. Subjects were provided with verbal encouragement throughout the task via the intercom. In the post exercise period, a $^{31}$P ISIS scan was performed to ascertain post exercise $^{31}$P metabolite concentrations. The blood pressure cuff was then released simultaneously with the initiation of the recovery scans which continued for 9-minutes.

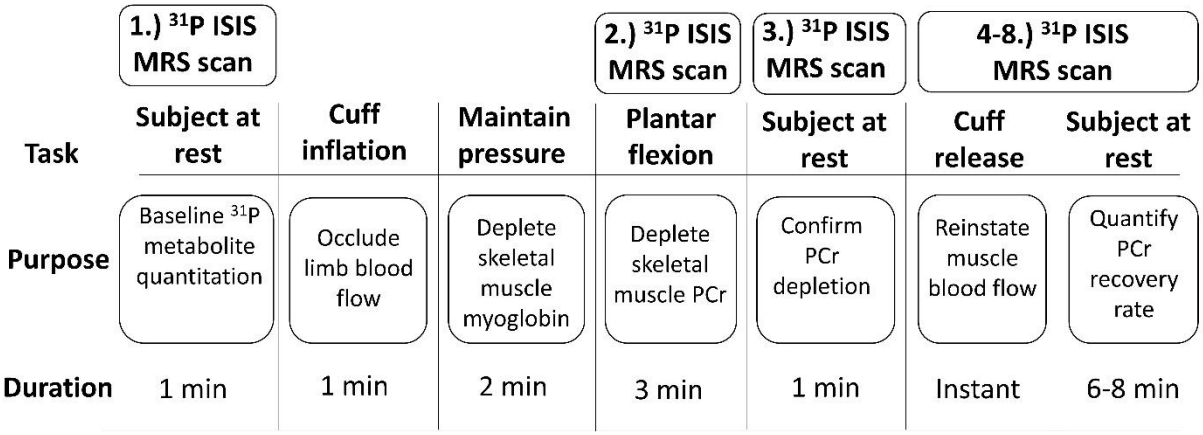



***Figure 3-23.*** *Schematic of $^{31}$P MRS muscle deconditioning protocol conceived and tested during the developmental period.*

The $^{31}$P MR spectra were analysed using jMRUI Beta 6.0 and Localised $^{31}$P-MR spectra were apodized to 10Hz with Lorentzian fitting. $^{31}$P MR spectra peaks including inorganic phosphate (Pi) phosphocreatine (PCr) and adenosine triphosphate (ATP) subunits $(\gamma - ATP, \alpha - ATP, ß - ATP)$ were fitted using the AMARES function with prior knowledge. The exercise kinetics for PCr was expressed relative to baseline signal amplitude. Cytosolic pH was calculated using the chemical shift difference $(\delta)$ between Pi and PCr peaks,

$$pH = pK + \log(\delta 1 - \delta 0 \div \delta 0 - \delta 2) \quad (1)$$

where p$K$ = 6.75, $\delta 1$ = 3.27, $\delta 2$ = 5.63.

Figure 3-24 demonstrates Pi and PCr kinetics across the ischaemic exercise task in the two healthy volunteers. Both Pi and PCr peaks were fit across the exercise protocol using the AMARES algorithm. This enabled quantitation of $^{31}$P metabolite kinetics during the transition from rest through to exercise recovery and to delineate differences in metabolic response between two healthy volunteers of divergent training status. For example, the $^{31}$P ISIS MR data displays total depletion of medial gastrocnemius PCr stores in Subject six, followed by rapid PCr resynthesis following reinstating of limb blood flow, such that muscle PCr signal amplitude overshoots baseline values at 01:48 following cuff removal. This remained constant throughout the nine-minute measurement period of exercise recovery. In contrast, the sedentary healthy volunteer (Subject 3) demonstrated reduced PCr consumption following the exercise-task and an inhibition of PCr recovery after limb blood flow was re-instated.

Whilst this may reflect genuine physiological differences between the two volunteers, resulting from training status, a number of problems were identified with this experimental protocol. Firstly, we found situations where $^{31}$P metabolite peaks were out of phase (Fig 3-25) resulting

in it not being possible to fit the relevant peaks and quantify $^{31}$P metabolite kinetics at this point of measurement. This was observed in a healthy volunteer during both mid-exercise (Fig 3-25. I) and crucially, during the initial exercise recovery (Fig 3-25. II). It was postulated that this motion related phasing issue during exercise recovery may be attributable to displacement of the lower limb, due to rapid deflation of the blood pressure cuff prior to exercise recovery. However, this phasing issue persisted during subsequent development experiments despite attempts to delay the initiation of $^{31}$P acquisition for periods ranging from 10 to 40 seconds post cuff release. The aim here was to initiate data acquisition after cessation of lower limb motion caused by the cuff release. However, it was not consistently possible to obtain $^{31}$P ISIS spectra of the medial gastrocnemius of adequate quality to enable $^{31}$P metabolite peak fitting at this crucial time point. All subsequent experiments resulted in consistent phasing issues to that shown in Figure 3-25 (II) regardless of the time delay utilised from cuff pressure release to $^{31}$P ISIS acquisition.

To further illustrate this issue, BOLD scans of the lower limb were obtained during the initiation of cuff inflation, two-minute maintenance period of ischemia and subsequent deflation. This revealed significant lower limb motion during the inflation and deflation of the blood pressure cuff. Figure 3-26 displays an approximate 20 mm of limb displacement of the calf in the Y-axis across the period of cuff inflation. This is maintained during ischemia and returns to baseline following cuff pressure release to reinstate limb blood flow. This is consistent with the persistence of phasing issues despite delaying data acquisition ≤40s post cuff release. It was established that a major contributor to the displacement of the lower limb was the placement of the blood pressure cuff at the distal thigh region, such that this pushed against the foam roller used to stabilise the lower limb during the experimental protocol, resulting in displacement of the lower limb (Fig. 3-26). This confirmed that the removal of the foam roller would aid in reducing lower limb displacement.

Figure 3-23 demonstrates our only successful acquisitions during this period. Concomitantly, these data highlight that a substantial portion of the biphasic PCr resynthesis occurs within this initial "fast" recovery period on the order of seconds (encompassed within the temporal resolution of the initial post-exercise $^{31}$P ISIS acquisition), secondary to a slower second phase (on the order of minutes). Thus, even if an adequate time delay had been identified that allowed the consistent acquisition of quality $^{31}$P ISIS spectra within this measurement period, we would have missed the majority of the $^{31}$P recovery kinetics that we wished to measure in order to infer on muscle mitochondrial function. Despite the clear ability of this protocol utilising $^{31}$P ISIS MRS to visually delineate (physiological state) when successful (Fig. 3-24) The motion related phasing issues together with the disparities in temporal resolution between these $^{31}$P ISIS acquisition and muscle PCr resynthesis meant that this $^{31}$P sequence was inappropriate for quantitatively assessing PCr recovery kinetics.

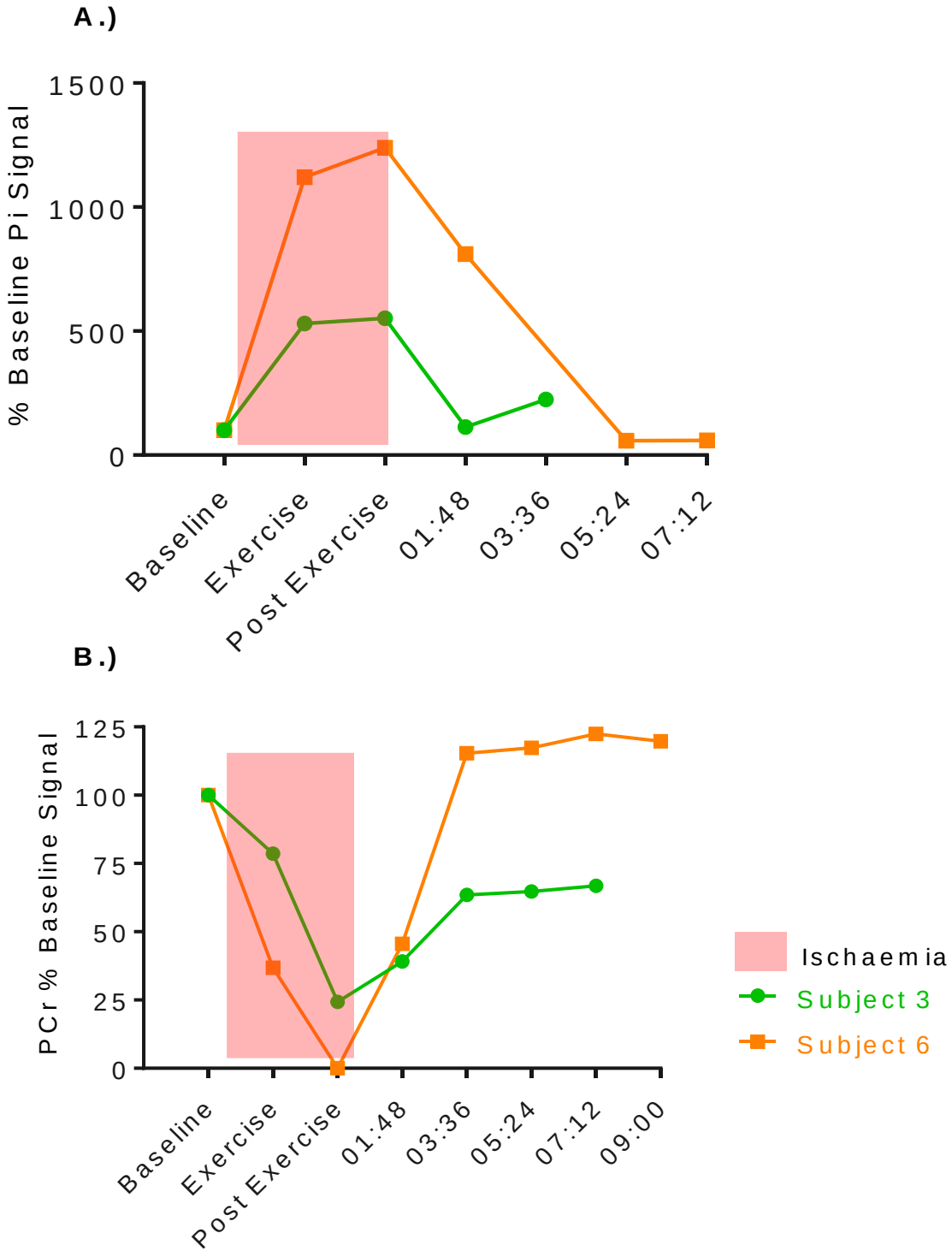



***Figure 3-24****. Pi (A) and (B) PCr kinetics across the ischaemic exercise task in two healthy subjects. Subject 3 is sedentary and Subject 6 is endurance trained. N.B. Post exercise time-point is ischaemic.*

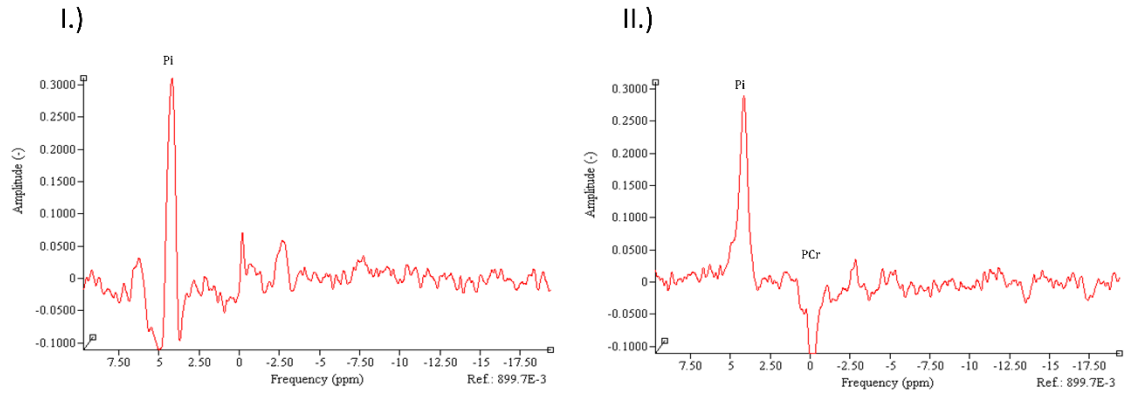



***Figure 3-25***. *$^{31}$P MRS ISIS spectra of the medial gastrocnemius during ischemic plantar flexion exercise (I) and during exercise recovery following removal of occlusion (II).*

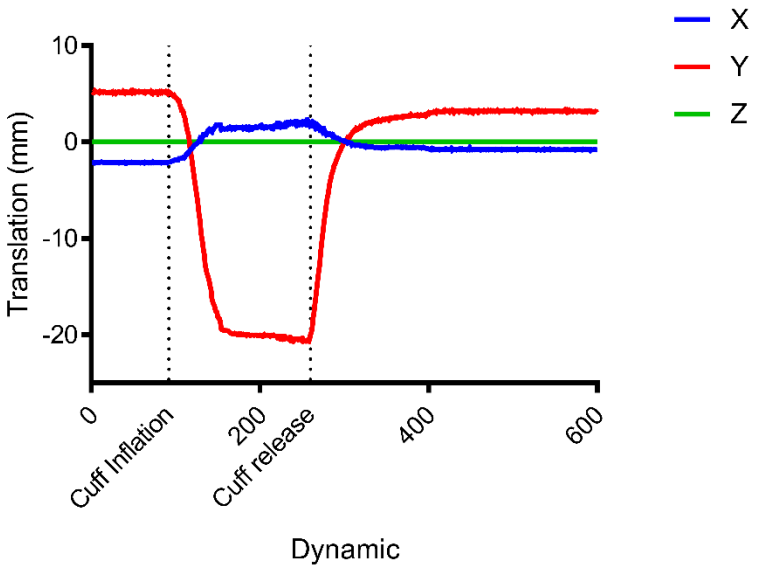



***Figure 3-26***. *Lower limb motion through the X, Y, Z planes across the ischemic plantar flexion exercise task. Significant lower limb displacement observed following the inflation of the blood pressure cuff, which stabilises during the occlusion period and returns back to baseline following release of the cuff.*

### 3.2.3 Optimised $^{31}$P MRS and $^{1}$H MRI protocol to study peripheral deconditioning

To negate the motion related issues and improve temporal resolution, the protocol was modified to use a non-localised pulse acquired $^{31}$P MRS acquisition with a temporal resolution of 8 seconds to allow the temporal tracking of PCr recovery kinetics. Two healthy volunteers were recruited to develop the non-localised pulse acquired $^{31}$P MRS protocol.

$^{1}$Hydrogen ($^{1}$H) DIXON scans were acquired to image the calf. Three $^{31}$P Image selected in-vivo spectroscopy (ISIS) scans were performed under resting conditions to ascertain muscle PCr concentrations within the medial gastrocnemius, soleus and peronei. A ten-minute resting state scan of the leg was then performed using $^{31}$P MRS in order to determine baseline $^{31}$P metabolite concentrations in the medial gastrocnemius. Upon completion, a 16-minute non-localized pulse-acquire $^{31}$P-MRS assessment followed, shimming over the medial gastrocnemius. $^{31}$P-MR spectra were collected under resting conditions for ~ 1 minute, the blood pressure cuff was then inflated to 250 mmHg and maintained at this level for 2 minutes. Ischaemic plantar flexions were then performed at a standardised 10W workload for three minutes or until contractile failure. Ischaemia was maintained post-exercise for 30 seconds after which lower limb blood flow was re-instated. $^{31}$P MRS acquisition continued during exercise recovery. Due to hardware issues during the first development experiment, we were unable to complete concentric plantar flexion exercise. Instead, in order to test the ability of the $^{31}$P non-localised acquisition to quantify $^{31}$P metabolite kinetics, repeated ischaemic contractions were performed isometrically by the healthy volunteer to elicit a metabolic

response.

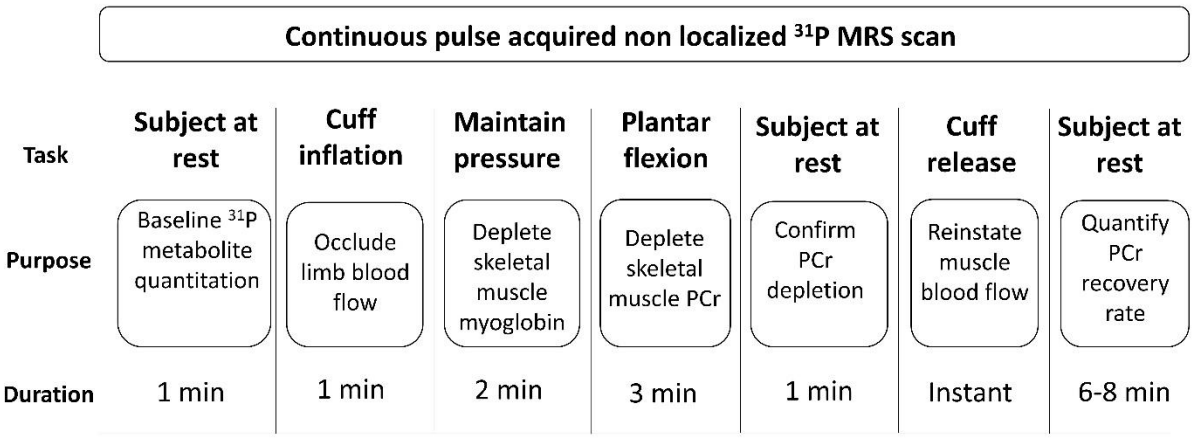



*Figure 3-27. Updated $^{31}$P MRS protocol to monitor muscle metabolite kinetics.*

Using the new protocol, it was possible to quantify $^{31}$P metabolite kinetics across the ischemic, isometric exercise task in the healthy volunteer, with a temporal resolution of 8 seconds. Figure 3-28 (I.) shows Pi and PCr kinetics across the isometric exercise task and the same data down sampled to the temporal resolution of our previously utilised $^{31}$P ISIS acquisitions. These data demonstrate a marked improvement in temporal resolution of the $^{31}$P metabolite kinetics (Fig, 3-28), independent of the ISIS related motion issues (Fig, 3-25 & 26), thus enabling appropriate quantitation of PCr recovery kinetics.

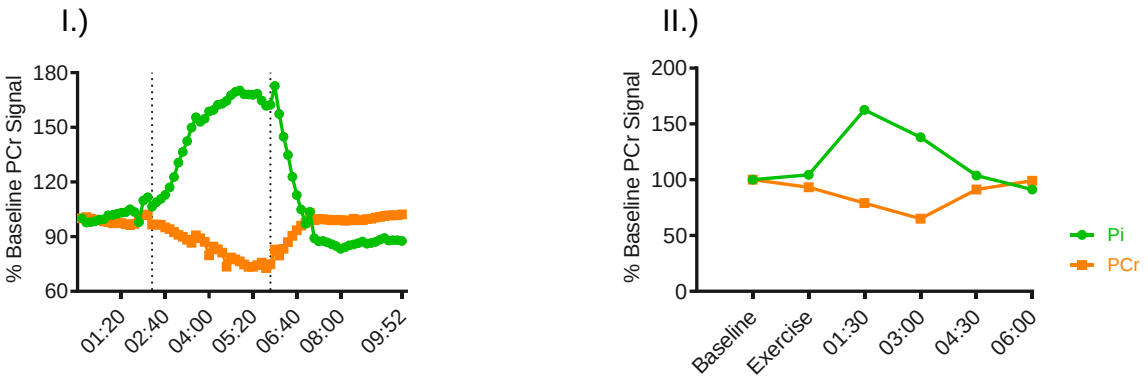



***Figure 3-28.*** *$^{31}$P MRS pulse-acquire spectra shimming over the medial gastrocnemius. (II.) The same series of spectra down sampled to the temporal resolution of the $^{31}$P ISIS acquisition.*

Whilst changing to a non-localised pulse acquired $^{31}$P MRS acquisition for the exercise task significantly improved our temporal resolution of PCr kinetics and negated the aforementioned motion related issues, it reduced spatial specificity of the acquired $^{31}$P signal to the medial gastrocnemius. This can complicate the use of post-exercise $^{31}$P kinetics to infer on muscle mitochondrial function. The use of a non-localised $^{31}$P-MRS sequence results in the volume of interest being determined by the sensitivity of the $^{31}$P surface coil. This lack of spatial specificity presents issues such as signal contamination from non-exercising muscle or acquiring $^{31}$P signal from muscle with a lower contribution to the mode of exercise. For example, the soleus PCr content may contribute to the acquired $^{31}$P signal using non-localised $^{31}$P MRS during plantar flexion, where the medial gastrocnemius is the main contributor, and often primary target [16, 17]. Thus, there is significant potential for contamination from either non-exercising muscle or muscle fibres contracting at a different intensity [17]. The volume of muscle in the $B_1$-field of the coil is also an important consideration. When the coil is placed on the target muscle during exercise using non-localised methods, the size and sensitivity of the surface coil will dictate the $^{31}$P signal intensity. Therefore, this approach is susceptible to issues arising from variations in subcutaneous fat mass, muscle mass and fibre composition

between subjects. This is of particular concern when assessing heterogeneous cohorts such as healthy volunteers and patients, where variations in these parameters are likely to exist. Figure 3-28 & 29 aid in visual representation of this problem. Figure 3-29 depicts calf MRI anatomy in the axial plane and associated position of the $^{31}$P surface coil where the deeper fibres can be visualised. Figure 3-30 provides an example of an axial DIXON scan in two subjects, one with a low and one with a high subcutaneous fat content in the lower limb. Thus, if comparing $^{31}$P metabolite kinetics between experimental groups with contrasting physiological characteristics such as fat content and muscle mass, interpretation of the measured metabolic parameters such as $k_{PCr}$, $Q_{max}$, $V_{PCr}$ etc is not straightforward, as the acquired $^{31}$P signal may have been acquired from a lower volume of muscle mass and from fibres with differential contractile and metabolic characteristics. Therefore, when using localised MRS, we acquired a baseline resting $^{31}$P MRS spectra at three separate locations across the calf anatomy. By doing this, we aimed to quantify the differences in PCr signal amplitude between these anatomic locations. By doing so, this would provide us with a proxy of the level of signal contamination from non-exercising muscle / fibres exercising at a different contractile intensity. Together with the assessment of whole muscle volume and fat fraction % from $^{1}$H DIXON scans of the calf, we hypothesised that these data would enable us to accurately identify any potential signal contamination. Further, it would also indicate whether differences in the volume of muscle mass within the sensitive

volume of the $^{31}$P surface coil were influencing the metabolic data obtained during our non-localised $^{31}$P MRS experiments.

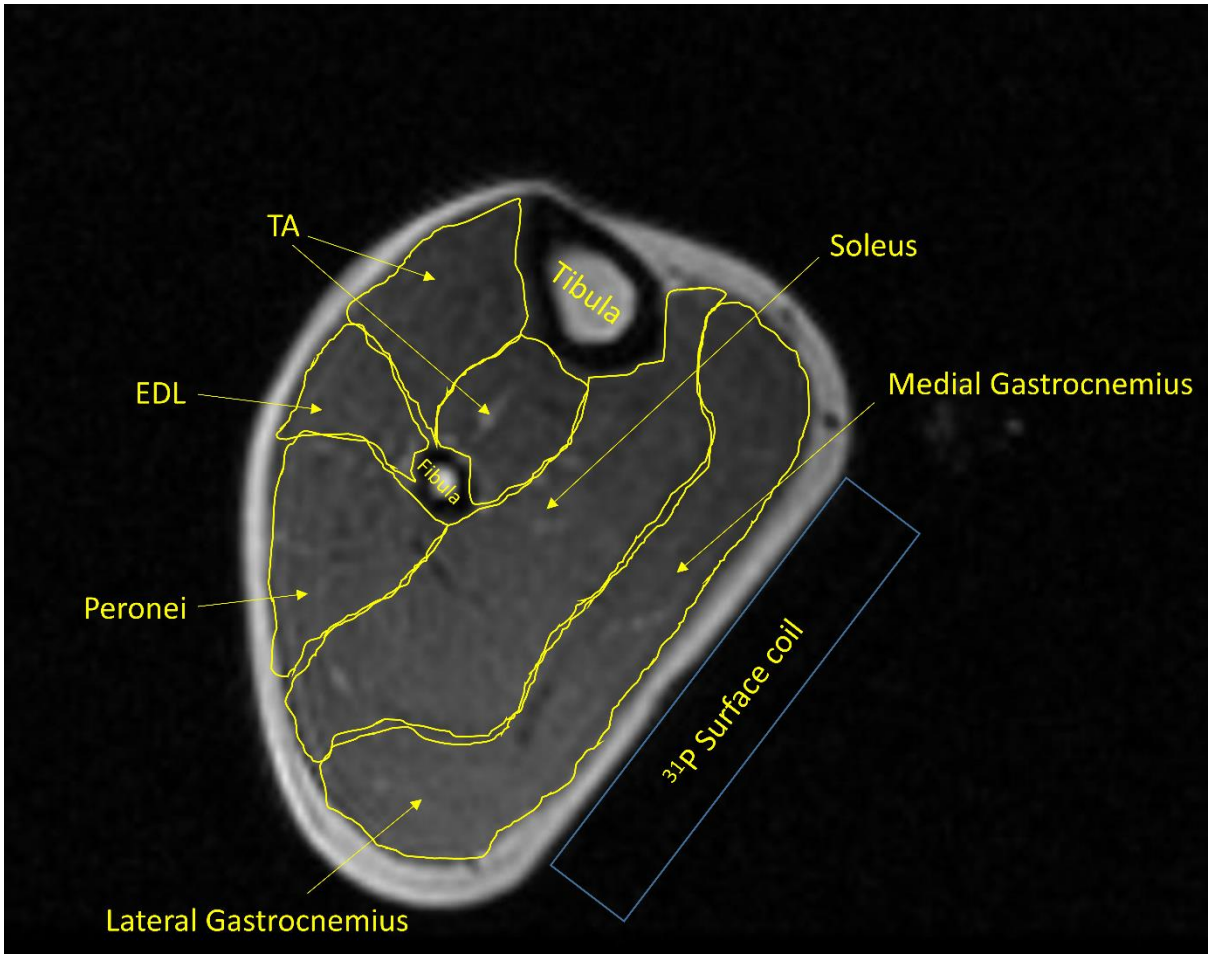



***Figure 3-29.*** *$^{1}$H MRI of human calf muscle in the axial plane demonstrating position of the $^{31}$P surface coil relative to calf anatomy.*

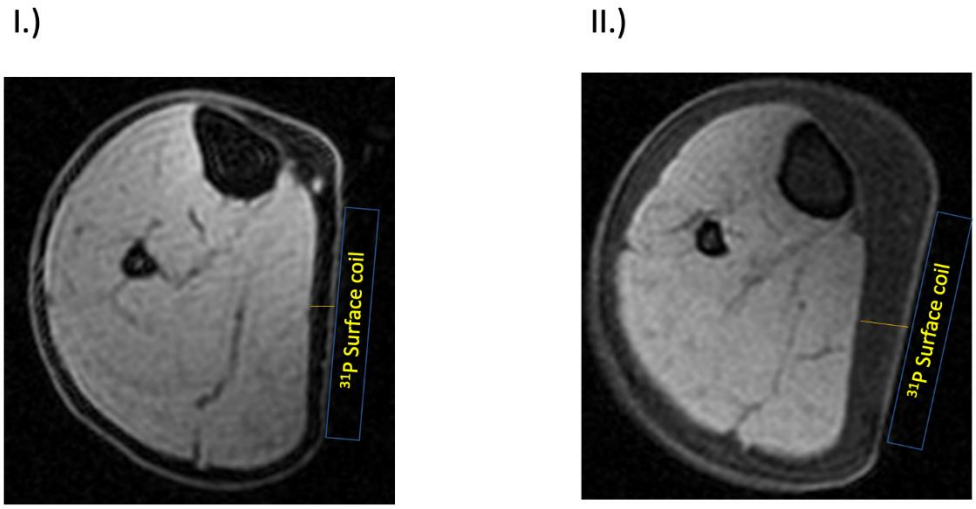



***Figure 3-30***. *Calf mDIXON's demonstrating position of the $^{31}P$ surface coil and differences in subcutaneous fat content between healthy control (I) and patient volunteers (II).*

Figure 3-31 shows a visual representation of the $^{31}P$ ISIS MRS spectra obtained from localising to the three distinct areas of calf anatomy under resting conditions. Quantitatively, there was a 29% reduction in PCr signal amplitude during $^{31}P$ ISIS MRS in the soleus (Location 2) relative to the signal acquired from localising to the medial gastrocnemius (Location 1). When localising to Location 3, The PCr signal amplitude was reduced to 47% of the medial gastrocnemius (Location 1) amplitude (Fig 4-31 & 32).

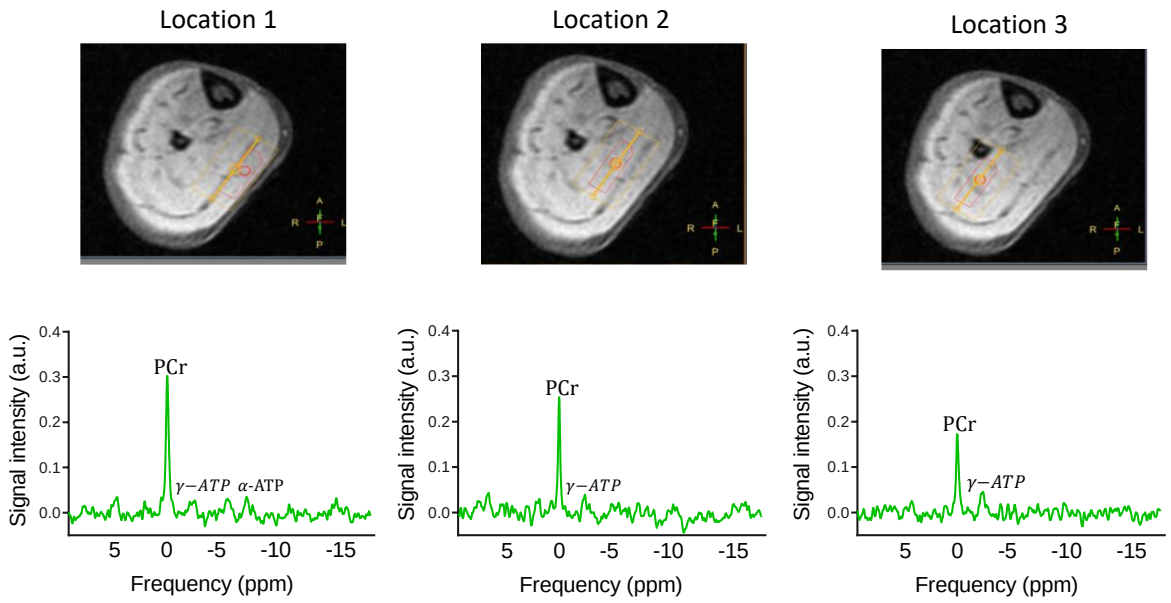



***Figure 3-31.*** *$^{31}$P MRS ISIS spectra localised to the medial gastrocnemius (Location 1) soleus (Location 2) and peronei (Location 3).*

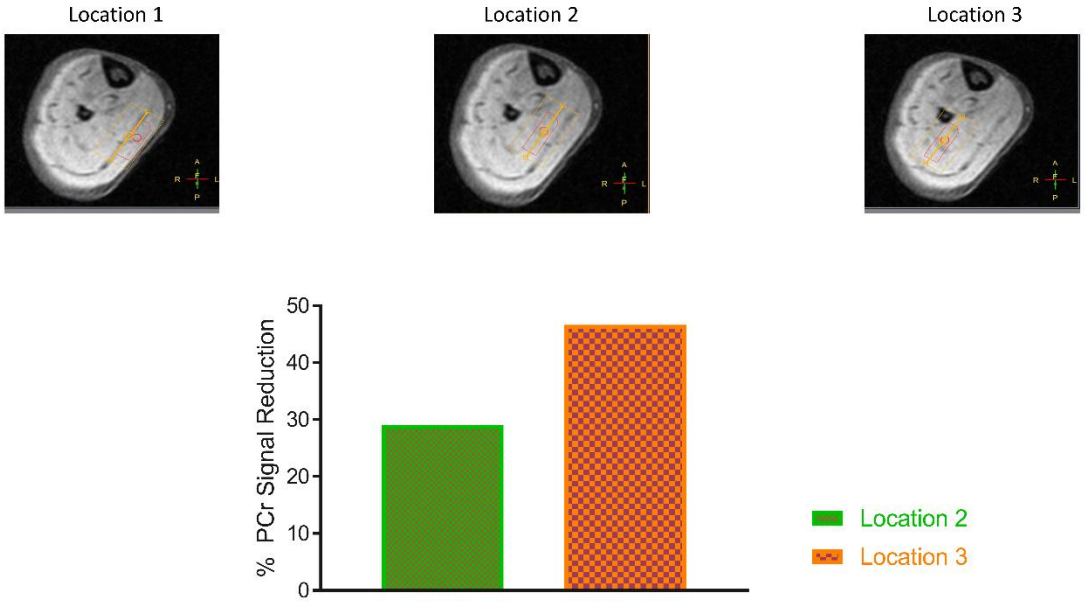



***Figure 3-32****. Percentage signal reduction from localising to the soleus and peronei relative to the medial gastrocnemius using $^{31}$P MRS ISIS.*

These data provide evidence of a substantial $^{31}P$ content in both the soleus and peronei muscles. As the $^{31}P$ signal acquired from localising to these muscle groups were 71 and 53% of the baseline PCr content in the medial gastrocnemius muscle. Based on these data, it is reasonable to assume that $^{31}P$ signal amplitude acquired during non-localised pulse acquired $^{31}P$ MRS acquisitions using the same 14cm $^{31}P$ Philips surface coil, will mostly represent contribution of the medial gastrocnemius. With the acquired $^{31}P$ signal representing a weighted sum of PCr recovery rates across various muscle fibres with varying mitochondrial and PCr content located within the volume of muscle mass in the $B_1$-field of the coil.

### 3.2.4 Standardising relative exercise intensity across volunteers

Review of the results from the initial three patient volunteers with the updated $^{31}P$ MRS protocol (Section 3.2.3), showed a significant variation in PCr consumption between volunteers using the standardised workload (Fig. 3- 33-34, Table 3-12). The coefficient of variation in PCr consumption between the three trials was 17.3%. Whilst PCr consumption during exercise tasks is known to be variable [18] the ischaemic component of this protocol essentially creates a closed metabolic compartment and negates the resynthesis of muscle PCr [19]. As a result, PCr consumption, intracellular acidosis and associated pH decrement, should be relatively well matched between volunteers. This ensures a comparable metabolic start point upon reinstating limb blood flow and that variation in the PCr resynthesis rate is not attributable to differential PCr consumption during exercise, as PCr consumption significantly impacts resynthesis kinetics [20].

Figure 3-33 displays the end-exercise $^{31}P$ MRS spectra following the standardised ischaemic exercise task in the initial three patient volunteers and associated variation in $^{31}P$ metabolite kinetics. Figure 3-34 (B.) shows both the PCr recovery curves derived from the raw and moving

average $^{31}$P MRS spectra in the three patient volunteers. This 17.3% coefficient of variation in PCr consumption arising as a result of the standardised, non-relative contractile intensity, creates an unfavourable, variable metabolic start point. This complicates the use of PCr recovery as a measure of muscle mitochondrial function between volunteers. As exercise intensity is not relative, the subsequent differences in muscle mass and fibre type recruitment will influence substrate utilisation, lactate formation and muscle pH. All of which influence the rate of PCr resynthesis [21]. It was thus decided to alter the protocol such that a standardised relative exercise intensity be employed across the remaining subjects.

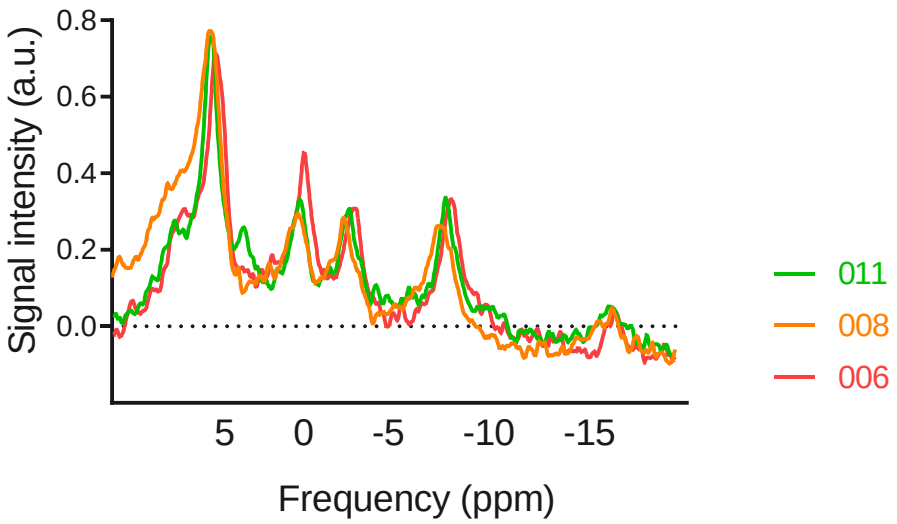



*Figure 3-33. End exercise non-localised pulse acquired $^{31}$P MRS spectra in three patient volunteers following standardised ischemic plantarflexions at 10W.*

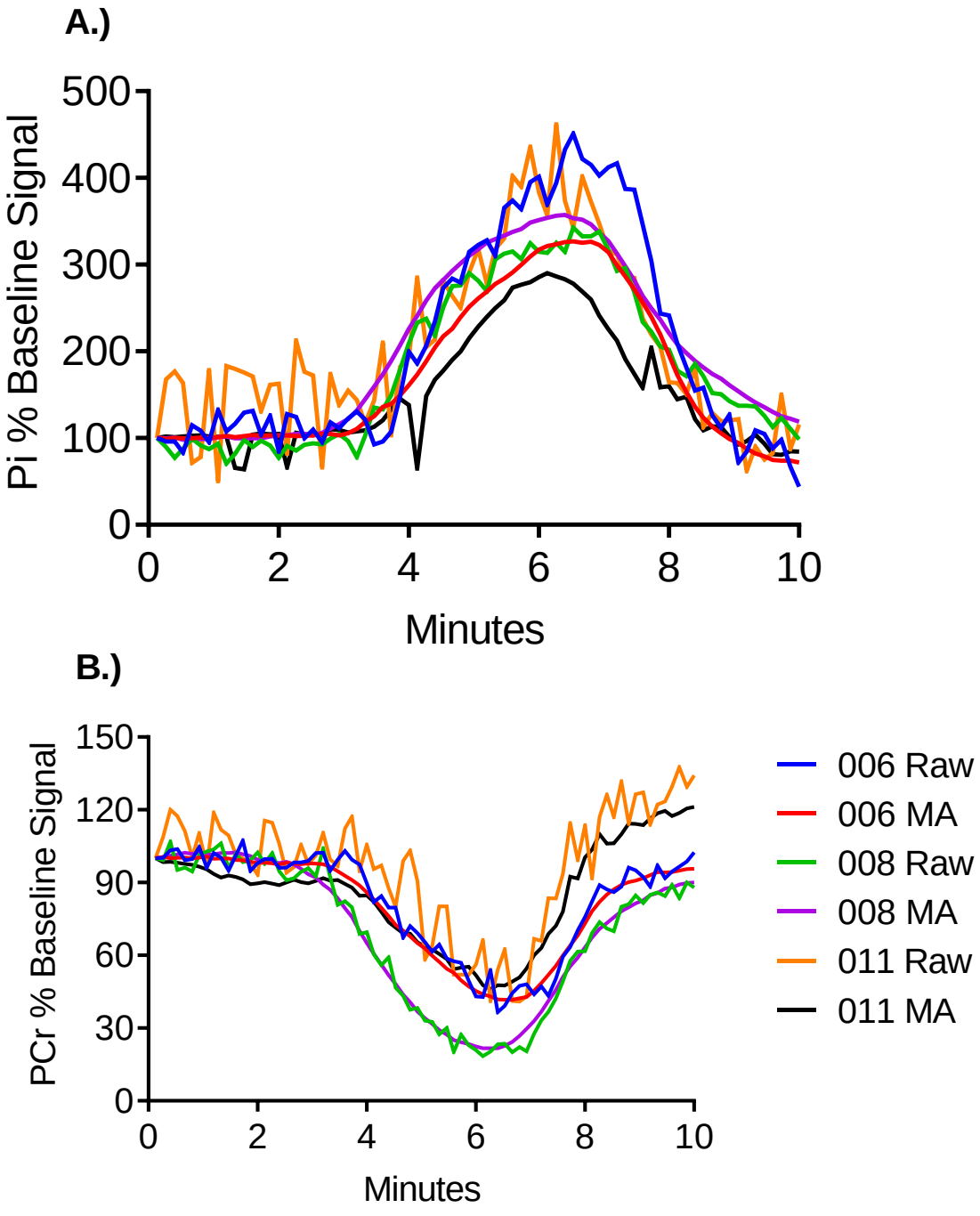



***Figure 3-34*** *(A.) Pi and (B.) PCr kinetics across the ischemic exercise task in the initially recruited patient volunteers. Curves are presented as "Raw" and "MA". MA is a ten-point moving average function performed on the raw data in MATLAB.*

| Age | BMI | PCr consumption (%) | PCr consumption CV (%) |
|---|---|---|---|
| 44 ± 16 | 24.5 ± 6.1 | 61.76 ± 10.68 | 17.30 |

*Table 3-12. Demographic data and the variation in PCr consumption in three patient volunteers.*

### 3.2.5 MVC determination and measurement of PCr content

Given the issues associated with the use of a non-relative exercise intensity (See previous Section 3.2.4), the relative contractile intensity was standardised in our experimental protocol. It was hypothesised that under lower limb ischaemia, this would enable us to tightly match both muscle mass and fibre type recruitment and associated PCr consumption, and muscle pH between volunteers. This would then allow mitochondrial function to be inferred based on $^{31}$P metabolite recovery kinetics.

The 3T compatible Trispect module enables volunteers to perform concentric and eccentric contractions. Additionally, the module contains a locking mechanism to facilitate the performance of isometric contractions. In the updated protocol, maximum calf strength of volunteers was measured and the relative exercise intensity during the $^{31}$P MRS protocol normalised to these MVC values, to standardise the contractile demand.

One healthy male volunteer was recruited for this development experiment. The volunteer lay supine on the MRI bed as previously described. Initially the volunteer was familiarised to plantar flexion exercise with a series of progressive isokinetic contractions beginning at a low RPE with minimal air pressure supplied to the device. This was gradually increased such that the RPE of the volunteer reached near maximal during the final contraction. After this, the volunteer was given a 3-minute recovery period before the maximum possible resistance level

was applied to the Trispect module, theoretically creating an immovable resistance. Using the PC in the console room, force recordings were started using the Ergospect software and the device was operated in manual mode, allowing air pressure supply to the Trispect device in 0.1 bar increments up to a maximum pressure of 5 bar. The volunteer was briefed on performing an MVC of the calf muscle. The volunteer was instructed to push against the fixed pedal as hard as possible for a period of 3 seconds. A 1-minute recovery period followed and the MVC was repeated a further two times. The volunteer was verbally encouraged throughout the brief contractions. The force kinetics across the exercise task were then plotted on a PC in the console room. The peak of the three MVC attempts (N) was taken as calf maximal strength. We then calculated 40 and 50% of the MVC force value (N). This was converted to bar using a conversion factor supplied by Ergospect engineers. We could then deliver the required relative exercise intensity whilst using the Trispect device in manual mode. The non-localised $^{31}$P MRS protocol (Section 3.2.3) was then performed at relative exercise intensities of 40 and 50% of the volunteers pre-determined MVC with an appropriate recovery interval between exercise tasks.


***Figure 3-35****. Calf strength assessment protocol prior to $^{31}P$ MRS at a standardised relative exercise intensity*

The subjects power output was higher across the exercise task at 50% MVC relative to the 40% trial (Fig 3-36, A). Consistently, there was a greater rise in Pi and a greater PCr consumption during the 50% trial relative to the 40% trial (Fig 3-36, B & C). End-excise PCr consumption was increased by 61% following the 50% MVC trial relative to the 40% trial (92.7 vs 57.6%). Figure 3-38 (A & B) shows Pi and PCr kinetics across the newly updated exercise task performed at 50% MVC. PCr resynthesis kinetics are also shown during recovery from the exercise task after reinstating limb blood flow (Fig 3-38, C) The data show a mono-exponential curve fitted to the recovery data which fitted the recovery kinetics well ($R^2$ value =0.97) and

returned a rate constant of PCr resynthesis ($k_{PCr}$) of 0.75 $min^{-1}$.

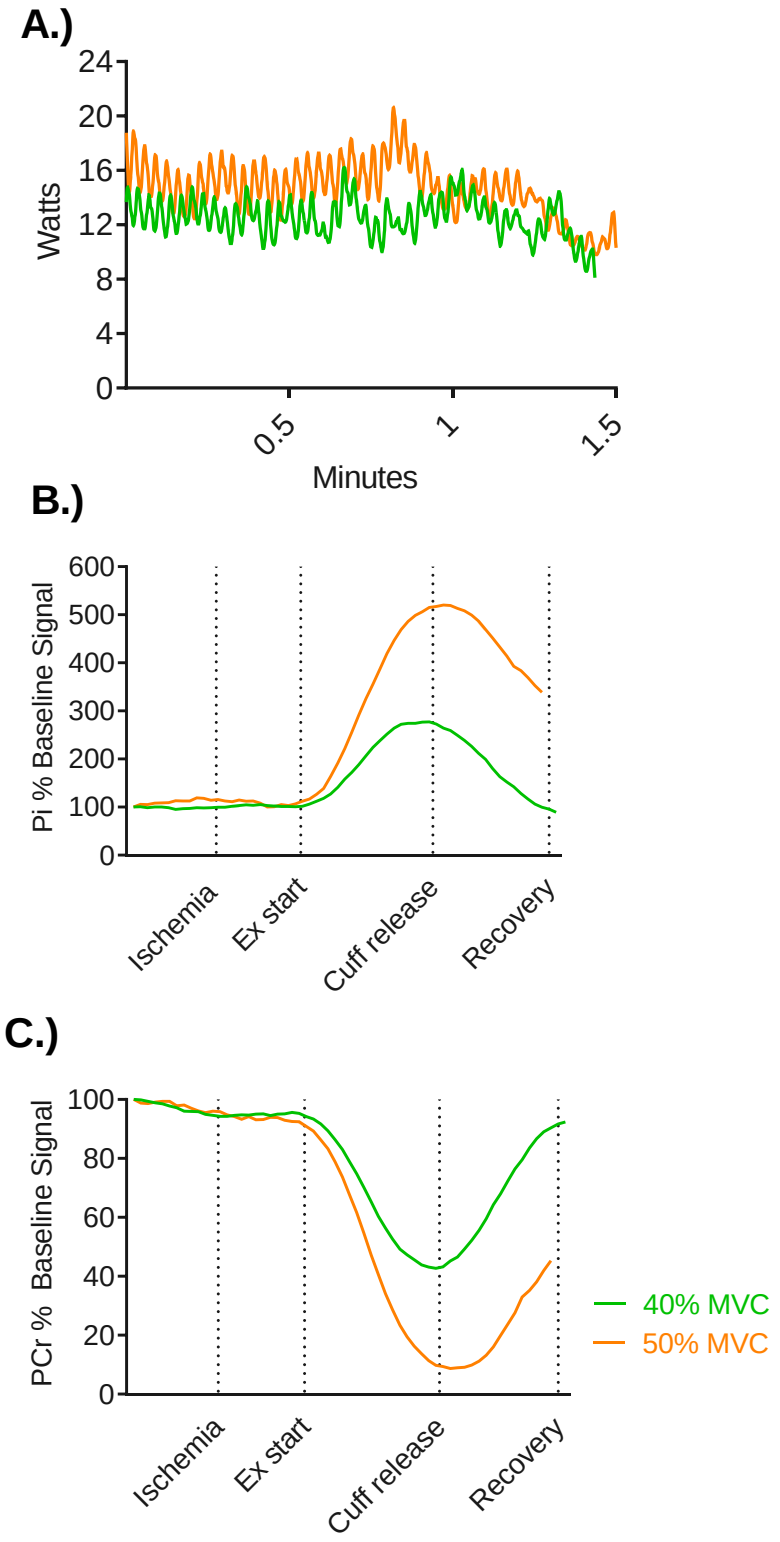



***Figure 3-36*** *A.) Power output, (B and C.) Pi and PCr kinetics during ischaemic plantar*

*flexion exercise at 40 and 50% MVC.* $^{31}$*P metabolite peaks fitted from spectra with a moving average function applied (x10 Non-localised spectra).*

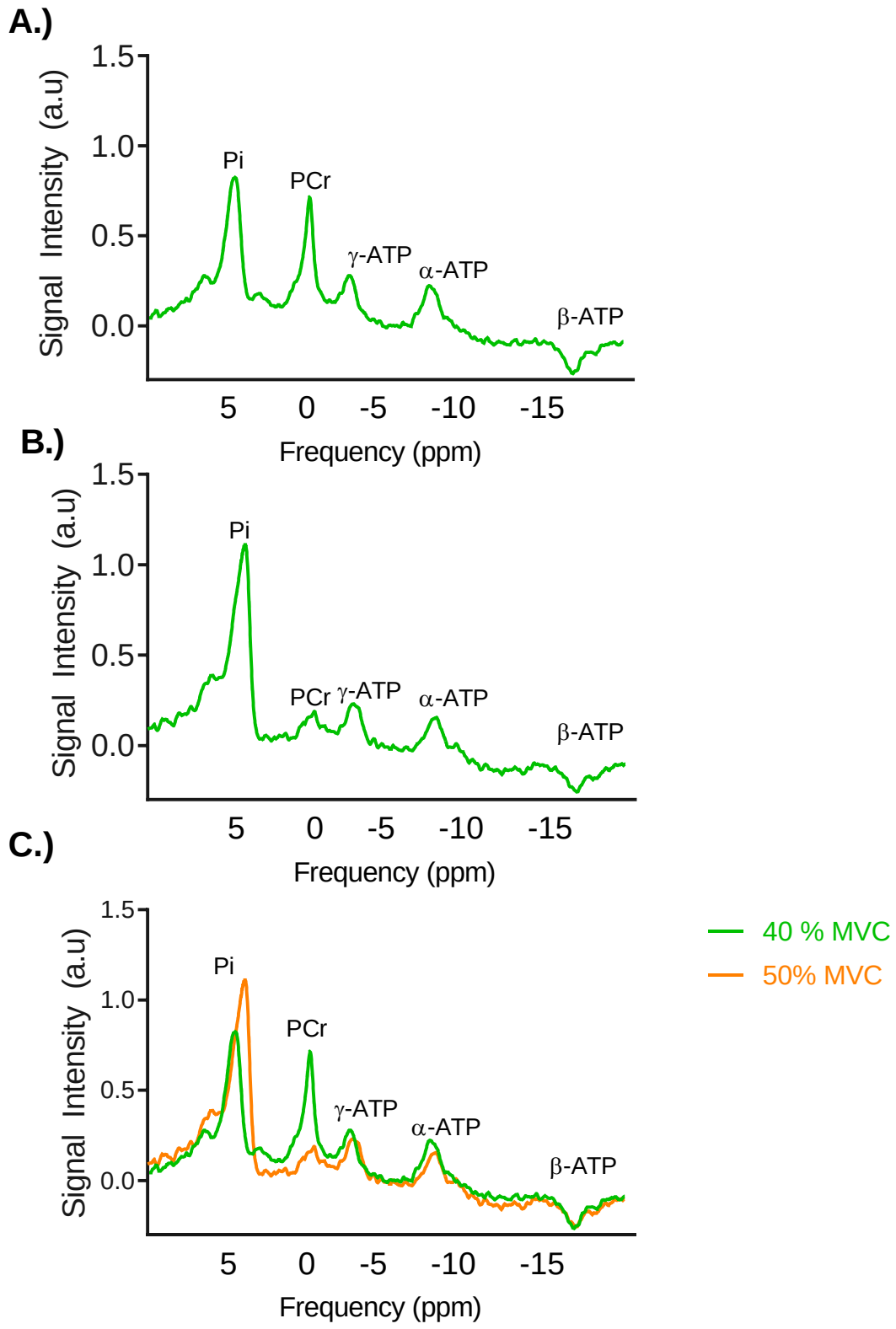



***Figure 3- 37*** *Pulse acquired $^{31}$P MRS spectra of human calf muscle at end exercise following (A.) repeated plantarflexions at 40% MVC and (B.) 50% MVC. (C.) 40 and 50% MVC spectra*

*superimposed. Single spectra presented are a binned average of 5 individual non-localised spectra acquired at contractile failure with ischemia maintained.*

| | **40% MVC** | **50% MVC** |
|---|---|---|
| Mean power output | 12.5 ± 1.4 | 14.6 ± 2 |
| PCr consumption (% of baseline concentration) | 57.57 | 92.69 |

***Table 3-13**. Mean power and end-exercise PCr consumption and pH concentrations following ischemic plantar flexion exercise at 40 and 50% MVC.*

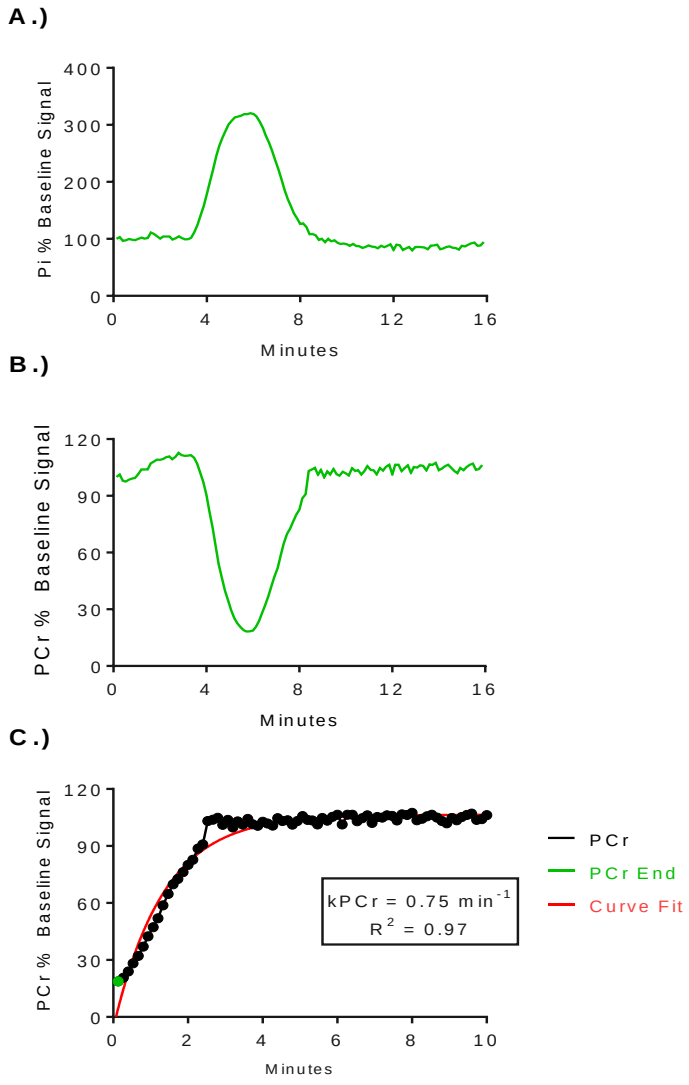



***Figure 3-38*** *Full Pi (A) and PCr (B) kinetics across an ischaemic plantarflexion exercise at 50% MVC (C) PCr recovery kinetics following reinstating of limb blood flow and associated rate constant of PCr resynthesis calculated using a mono-exponential function.*

Considering these data, we chose to update our $^{31}$P MRS protocol and standardised the relative exercise intensity to 50% MVC. These data showed substantial PCr depletion in response to plantar flexion at this contractile intensity (Fig 3-36-37 & Table 3-13) and through the associated elevation in ADP concentrations, would sufficiently stimulate mitochondrial

respiration and subsequent ATP synthesis, relative to the lower intensity contractions. We also added three $^{31}$P ISIS MRS scans across three muscle groups to the main experimental scan card in order to ascertain the potential for $^{31}$P signal contribution from skeletal muscle other than the medial gastrocnemius. These developmental data paired with existing knowledge on dynamic non-localised pulse acquired $^{31}$P MRS also confirmed the need for quantitation of whole calf muscle volume and percentage of fat fraction from the $^{1}$H DIXON scans already present on the exam card. It was arranged that a fellow PhD student (Rosemary Nicholas) would take a lead on the analysis of these compositional data derived from $^{1}$H MRI. Figure 3-39 shows a schematic of the finalised $^{31}$P MRS experimental protocol, associated analysis steps and the experimental data derived from these analyses.

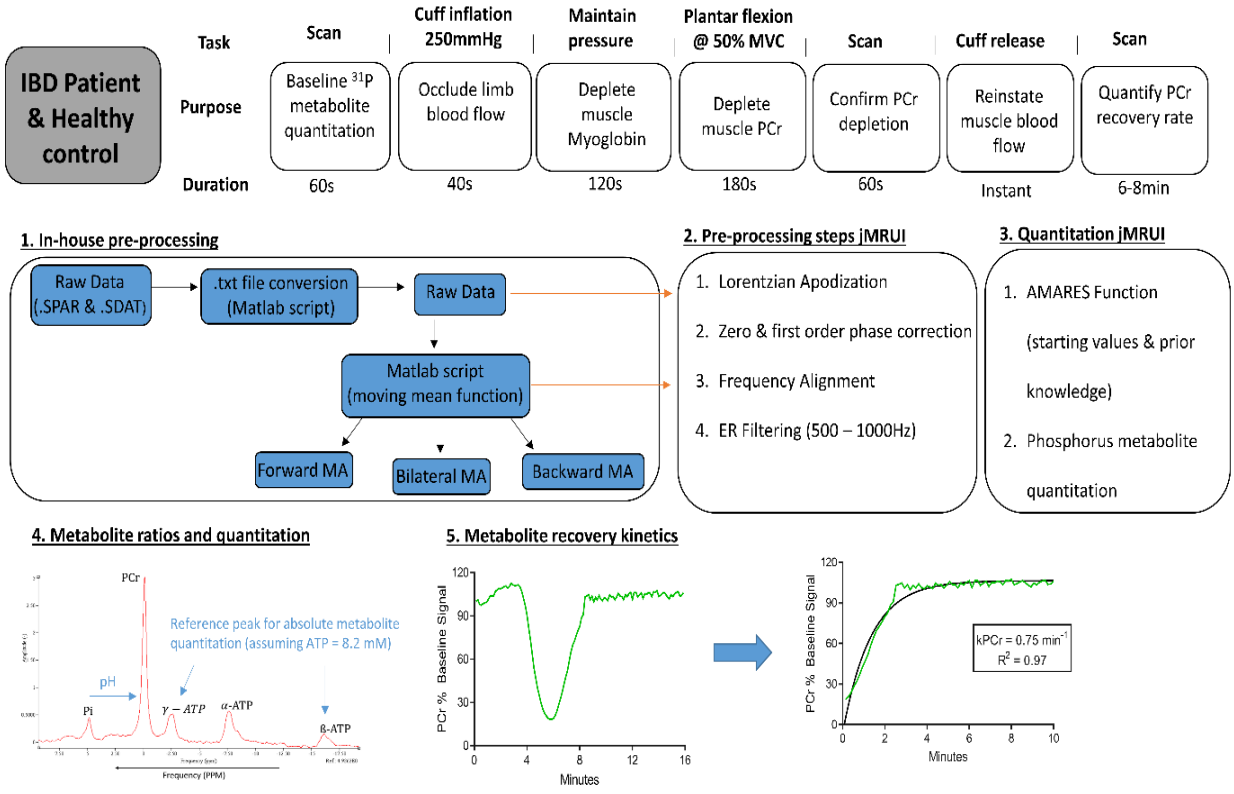



***Figure 3-39**. Finalised experimental $^{31}$P MRS protocol and pre/post processing steps.*

### 3.2.6 Saturation correction

$^{31}$P MRS can be used for the absolute quantitation of $^{31}$P metabolite concentrations in skeletal muscle using a range of approaches. A common and simplistic approaches is to estimate muscle PCr by using muscle ATP concentrations as an internal reference standard. This is based on the assumption that ATP remains constant on exercise [22]. Therefore, providing $^{31}$P MRS peak amplitudes have been corrected for partial saturation effects, muscle PCr concentrations can be indirectly estimated from the ratio of (PCr/ATP) [23]. This is a popular approach most likely due to the simplicity and practicality relative to other experimental methods such as using tissue water measured via $^{1}$H MRS or a $B_1$ field map.

Taking into consideration the duration of MRI scanning our study volunteers were required to undergo within a single day, it was decided that ATP would be used an as internal reference standard to minimise the burden on volunteers. Given that available data using "uncalibrated MRS" in human calf muscle for $^{31}$P metabolite quantitation has generally produced results comparable to that of more complex localisation techniques [23], it was decided that this would be the best experimental approach. In order to calculate absolute metabolite concentrations, resting state $^{31}$P MR spectra were acquired under partial saturation and fully relaxed conditions in order to generate a saturation correction factor.

Data were collected on a Philips 3T Achieva as previously described with a 14 cm Philips $^{31}$P coil, in three volunteers. The experimental scan card contained the dynamic non-localised pulse acquired MRS acquisition with a 4s TR acquired to quantify signal PCr concentrations in the medial gastrocnemius (Fig 3-31 and 3-32). To correct for partial saturation across these scans and enable accurate estimation of $^{31}$P metabolite concentrations, a series of five non-localised FID $^{31}$P MRS spectra were acquired with a TR of 4 s and then 15 s (fully relaxed). Saturation factors for each $^{31}$P metabolite were generated by dividing the $^{31}$P peak amplitude obtained during a long TR by that acquired during a short TR. The peak amplitudes obtained during the

non-localised $^{31}$P exercise protocol were then multiplied by this correction factor to correct for partial saturation effects.

The mean amplitude ratios for $^{31}$P metabolites obtained using a TR of 4 and 15 s using a non-localised $^{31}$P sequence are shown in table (3-14).

| **FID** | $\boldsymbol{\gamma - ATP}$ | **PCr** | **Pi** |
|---|---|---|---|
| Mean | 1.12 | 2.14 | 2.29 |
| SD | 0.25 | 0.22 | 0.41 |

***Table 3-14.*** *Non-localised $^{31}$P metabolite amplitude ratios (15s TR / 4s TR).*

### 3.2.7 $^{31}$P MRS Repeatability

The repeatability of $^{31}$P MRS measurements was also studied. Previous data demonstrate a coefficient of variation of 5.8 and 7.8% for PCr and Pi respectively in human quadriceps muscle, whilst intracellular pH was reported at 0.3% [24]. The coefficient of variation for PCr concentrations calculated from a non-localised FID $^{31}$P MRS sequence in the human calf at 3T ranged from 6-7% when assessed individually in trained volunteers across ten experiments [25].This is consistent with the 8% CV of PCr concentrations in the calf muscle of 15 trained male volunteers [26].

A healthy female volunteer was recruited to undergo two resting state $^{31}$P MRS scans. Data were collected on a Philips 3T Achieva as previously described. The experimental scan card contained the dynamic non-localised pulse acquired MRS acquisition with an 4s TR to quantify signal PCr concentrations in the medial gastrocnemius (Fig 3-28). To assess the repeatability

of these $^{31}$P data, this experimental protocol was repeated the following day in the same volunteer under identical conditions.

Table 3-15 shows the $^{31}$P metabolite signal intensities (M ± SD and CV) for non-localised $^{31}$P MRS between the two experiments across baseline, end-exercise and end recovery time points. The signal intensity (M ± SD) during resting state $^{31}$P spectra with long and short TR during non-localised and ISIS acquisitions are reported alongside the corresponding coefficient of variation for each.

| **Acquisition** | **$\alpha$-ATP** | | **$\gamma - ATP$** | | **PCr** | | **Pi** | | **pH** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | M ± SD | CV | M ± SD | CV | M ± SD | CV | M ± SD | CV | M ± SD | CV |
| FID 5-point average Baseline | 0.043 ± 0.031 | 70 | 0.039 ± 0.018 | 47 | 0.107 ± 0.008 | 7.49 | 0.038 ± 0.005 | 13.66 | 7.08 ± 0.13 | 1.88 |
| FID 5-point average End-exercise | 0.040 ± 0.025 | 63 | 0.043 ± 0.016 | 36 | 0.025 ± 0.013 | 54.02 | 0.113 ± 0.016 | 14.09 | 6.37 | N/A |
| FID 5-point average End-recovery | 0.038 ± 0.025 | 65 | 0.047 ± 0.003 | 6 | 0.098 ± 0.016 | 16.45 | 0.029 ± 0.001 | 5.12 | 7.24 ± 0.19 | 2.58 |
| FID 30s TR | 0.045 ± 0.033 | 74.55 | 0.041 ± 0.024 | 59.57 | 0.235 ± 0.01 | 4.35 | 0.082 ± 0.007 | 8.88 | 7.11 ± 0.01 | 0.11 |

***Table 3-15.*** *Repeatability of phosphorus metabolite peak amplitude obtained during the same acquisitions performed in one healthy volunteer on different days. Data presented are the M ± SD and CV for signal amplitude values and the corresponding coefficient of variation.*

These repeatability data for during identical experiments performed one day apart show that the variability in PCr and Pi peak amplitudes are similar to aforementioned literature values ≈ 8% [25, 26]. The high variation noted between the two-end exercise states of the experiments was due to an error from the researcher which unfortunately led to premature cessation of the

ischemic contraction in the first $^{31}$P MRS experiment. Thus, when re-performed the following day, greater PCr depletion occurred and led to high variation between the two end-exercise PCr measurements. The reproducibility of the ischemic exercise component of the $^{31}$P MRS protocol between volunteers is demonstrated in the later experimental chapter 7. Here, PCr consumption is shown to be tightly matched between volunteers given the standardized, ischemic contraction. Thus, despite this experimental error, the repeatability of basal state $^{31}$P MRS measurements has been established, which paired with the later experimental data demonstrating tightly matched PCr consumption, demonstrates the general efficacy of the measurements.

### 3.2.8 $^{31}$P MRS Post-processing

To optimize the pre-processing and subsequent peak fitting of the $^{31}$P MRS spectra, various pre-processing steps were applied to the same set of data to identify the most appropriate approach. PCr and Pi kinetics quantified across the exercise task were plotted as "Raw" data whereby the $^{31}$P peaks from non-localised $^{31}$P MRS spectra (TR 4s) were fit and plot as a function of time. The same set of data was then pre-processed in MATLAB using the 'Trailing moving average' of vector and the 'Centred moving average' of vector function. The Trailing moving average function was performed such that spectral averaging began in a "forward" and "backward" direction. The Centred moving average of vector function, averages data points bilaterally (Figure 3- 41-43). The PCr recovery curves generated from peak fitting of the raw spectra and those pre-processed in MATLAB were then plot and fit using a single exponential function using GraphPad Prism.

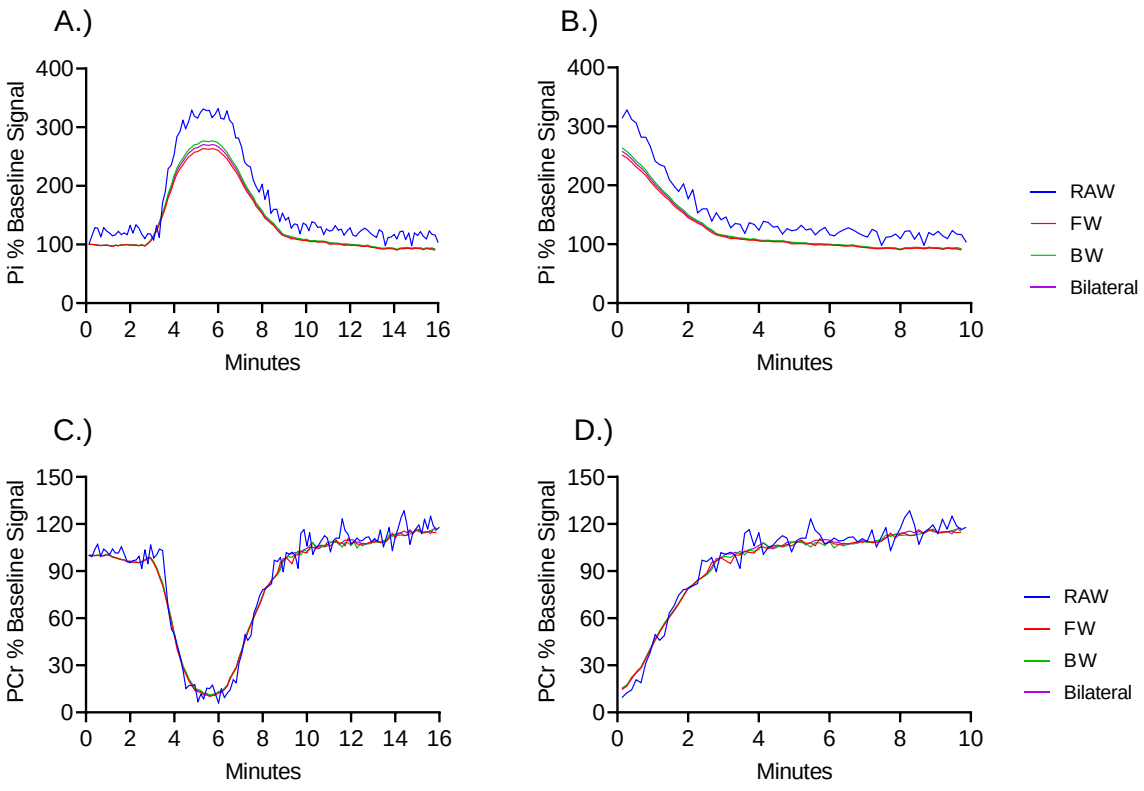



***Figure 3-40.*** *Pi and PCr kinetics across an ischaemic exercise task derived from Non-localised $^{31}$P MRS. Metabolite curves are presented are those derived from raw spectra (TR 4s) and a number of moving average functions performing in MATLAB on the raw data (x10 spectra TR8s). Forward (FW), Backward (BW) and Bilateral relate to the direction of spectral averaging.*

| Fitting variables | Raw | Trailing average FW | Trailing average BW | Centred average |
|---|---|---|---|---|
| Y0 | -13.4 | -4.059 | -3.457 | -4.469 |
| Plateau | 116.7 | 113.8 | 113.7 | 114.4 |
| $k_{PCr}$ | 0.62 | 0.58 | 0.59 | 0.59 |
| $R^2$ | 0.96 | 0.99 | 0.99 | 0.99 |
| Absolute Sum of Squares | 2584 | 670 | 702 | 733 |

***Table 3-16****. Fitting variables from a mono-exponential function applied to the PCr recovery curves displayed in figure 3-40.*

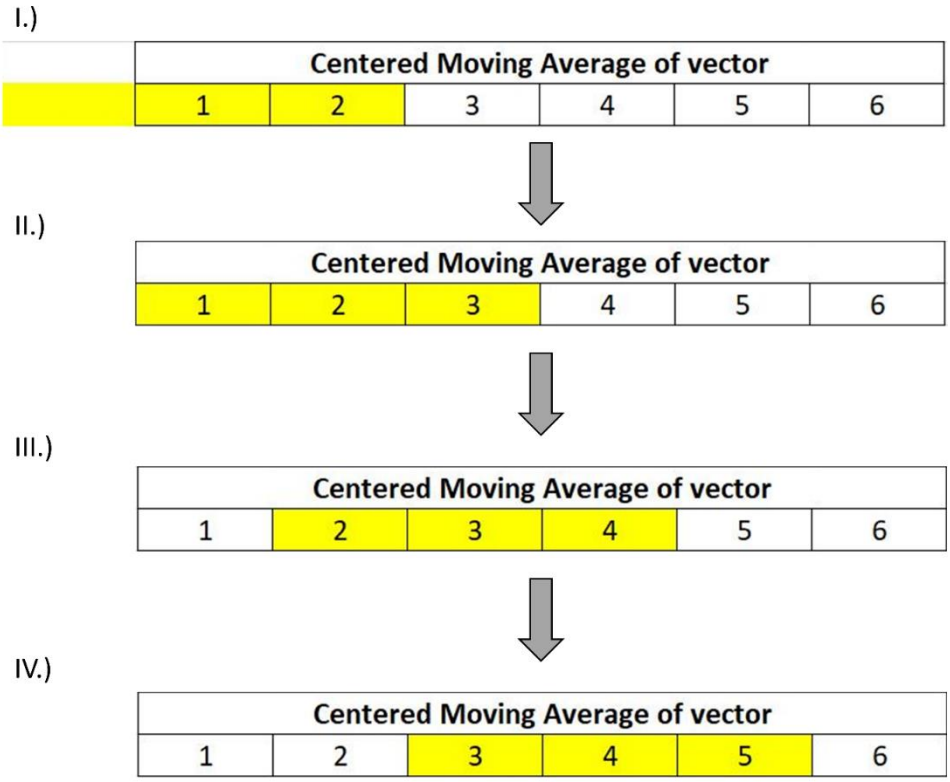



***Figure 3-41**. A graphical representation of the centred moving average of vector function used in MATLAB to perform averaging of the $^{31}$P MRS spectra across the exercise task. Numbers represent theoretical $^{31}$P MRS spectra number and the yellow highlights show the dynamic of $^{31}$P MRS averaging across time. Function referred to as "Bilateral" in text.*

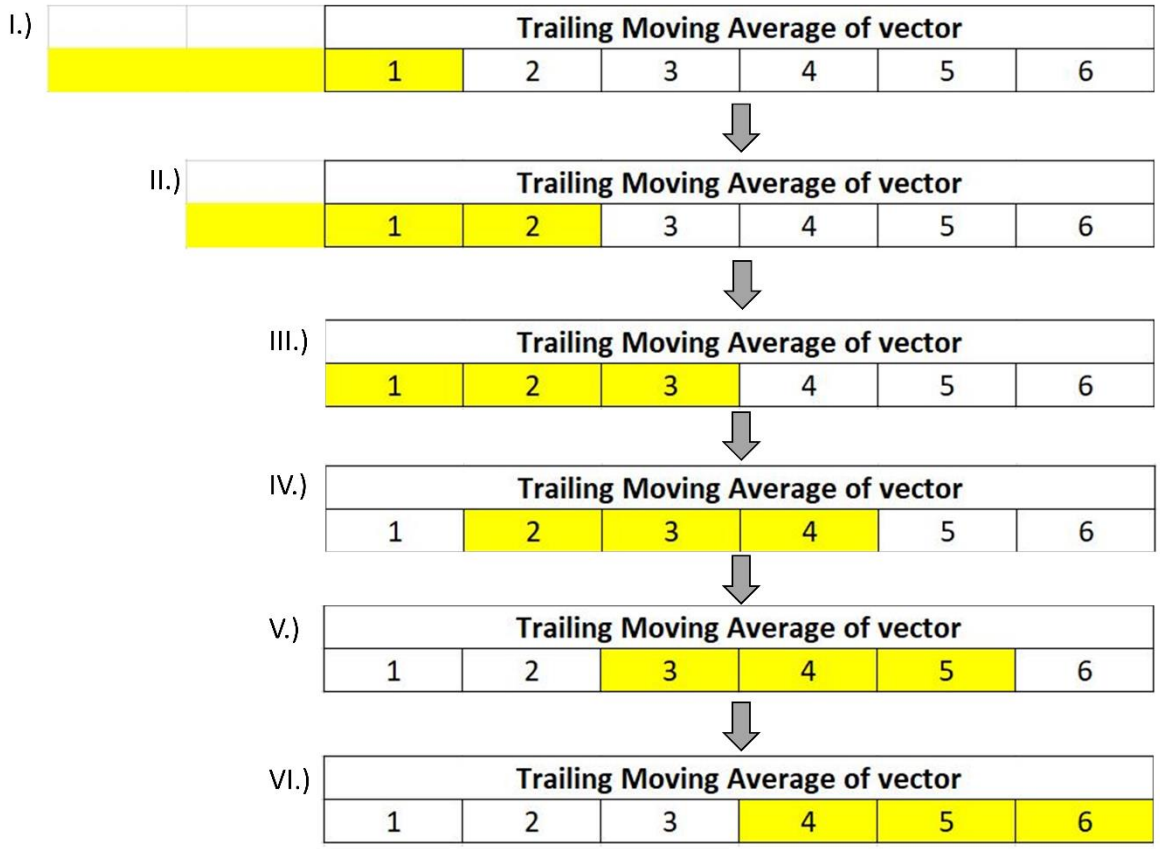



***Figure 3-42.*** *A graphical representation of the trailing moving average of vector function used in MATLAB to perform averaging of the $^{31}$P MRS spectra across the exercise task. Numbers represent theoretical $^{31}$P MRS spectra and the yellow highlights show the dynamic of $^{31}$P MRS averaging across time. Function referred to as "BW / Backward" in text.*

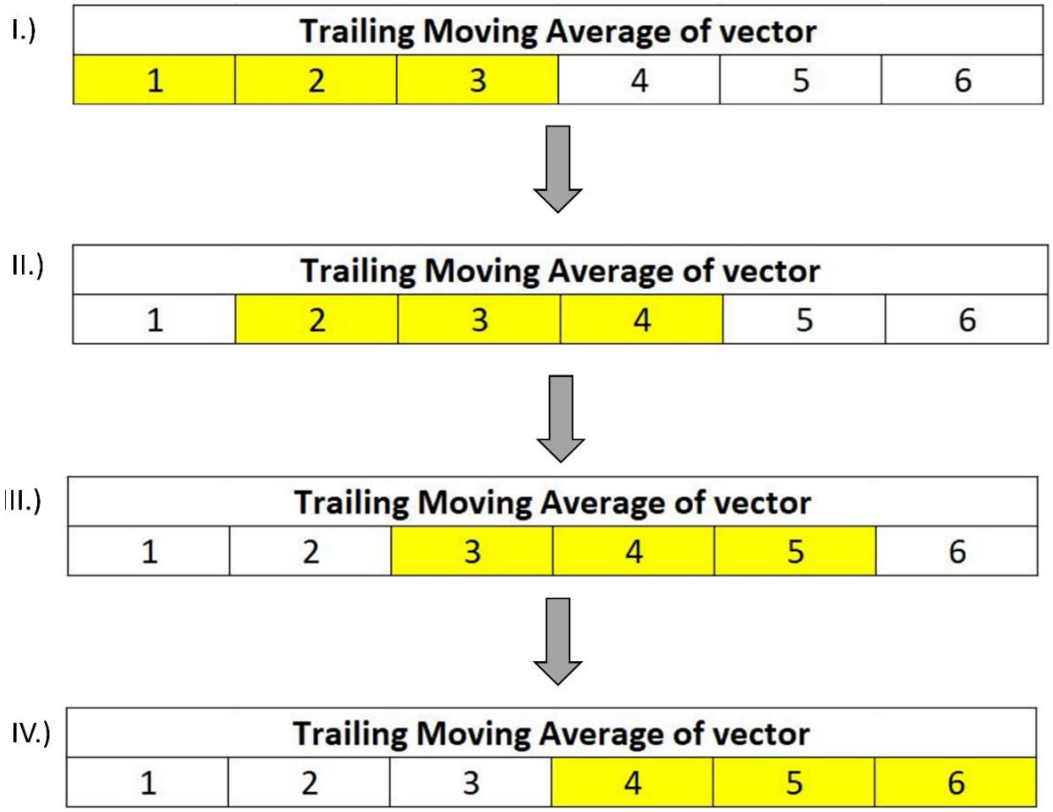



***Figure 3-43.*** *A graphical representation of the trailing moving average of vector function used in MATLAB to perform averaging of the $^{31}$P MRS spectra across the exercise task. Numbers represent theoretical $^{31}$P MRS spectra and the yellow highlights show the dynamic of $^{31}$P MRS averaging across time. Function referred to as "Forward / FW" in text.*

The results demonstrated a marked improvement in the quality of the curve fit when the $^{31}P$ peaks were fitted on spectra that had been pre-processed using a moving average function. This was evident from an improvement in the $R^2$ value and a reduction in the absolute sum of squares between the Raw and averaged data (Table 3-17). The quality of the curve fit was consistent across all averaged data, regardless of the function used. Therefore, the moving average function was utilised on the experimental data in order to improve the SNR of the $^{31}P$ spectra and subsequent ability to fit the $^{31}P$ spectral peaks.

Next, the effect of applying an ER filter to the $^{31}P$ MRS spectra was assessed. Prior to quantifying the $^{31}P$ peaks using the AMARES function in jMRUI. The PCr recovery curves from the Raw $^{31}P$ spectra, together with those derived from multiple averaging functions applied during pre-processing in MATLAB, were assessed both with and without application of an ER filter in jMRUI.

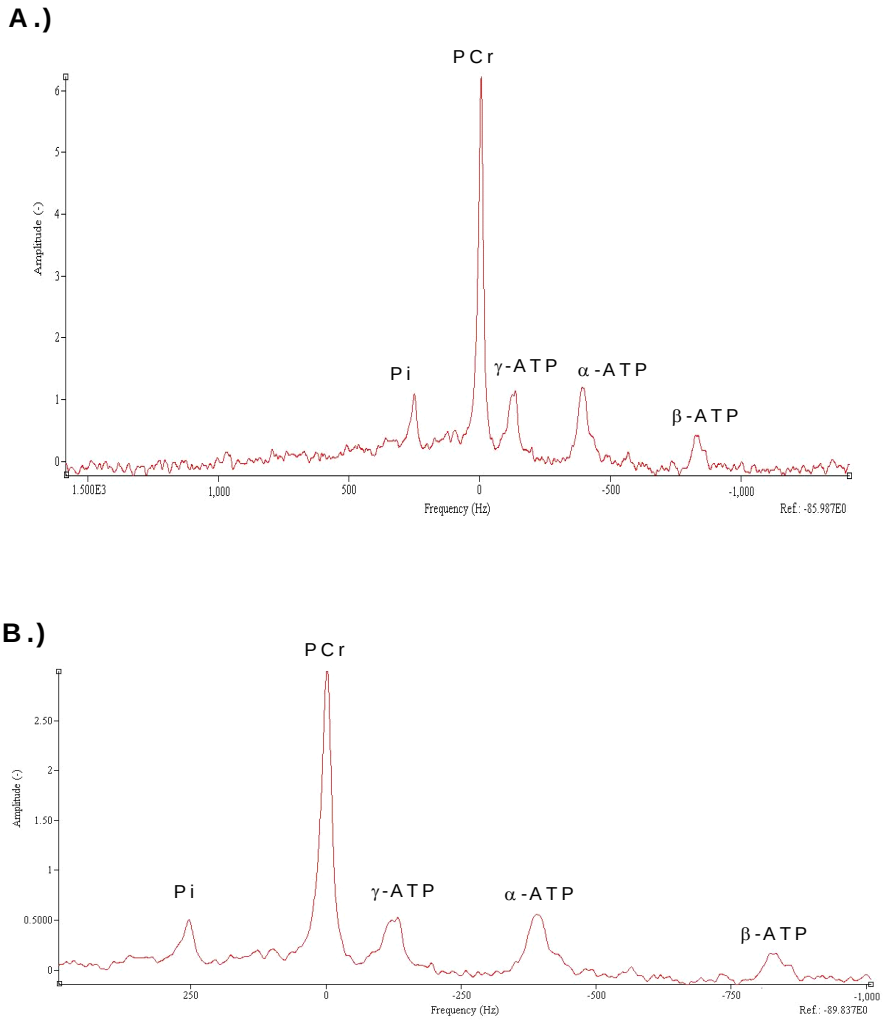



***Figure 3-44.*** *Displaying a non-localised $^{31}$P MRS spectra of human calf muscle (II.) The same $^{31}$P MRS spectra with an ER filter applied between 500 and -1000 Hz to aid in $^{31}$P metabolite peak fitting.*

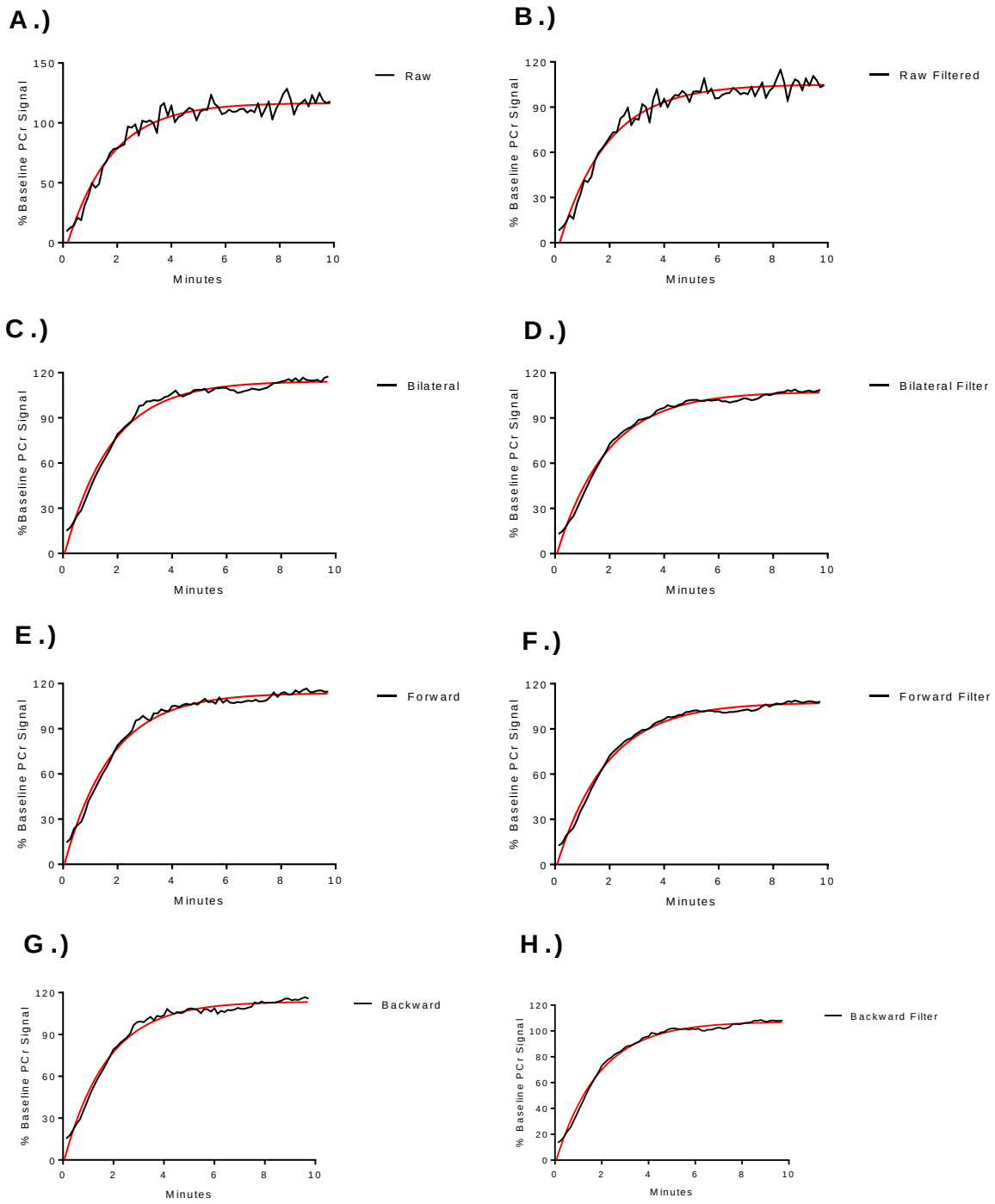



***Figure 3-45.)*** *Displaying PCr recovery curves and mono-exponential curve fit across the entire range of moving average functions both with and without application of an ER filter during pre-processing. PCr recovery curve from the raw* $^{31}$*P MRS spectra is also included.*

| Fitting Variables | Raw | Raw | Bilateral | Bilateral | Forward | Forward | Backward | Backward |
|---|---|---|---|---|---|---|---|---|
| | No Filter | Filter | No Filter | Filter | No Filter | Filter | No Filter | Filter |
| Y0 | -13.4 | -10.82 | -4.469 | -4.204 | -3.457 | -4.309 | -3.457 | -3.363 |
| Plateau | 116.7 | 105.2 | 114.4 | 107.5 | 113.8 | 107.6 | 113.7 | 107.3 |
| $k_{PCr}$ | 0.6156 | 0.5717 | 0.5873 | 0.5437 | 0.5821 | 0.5398 | 0.586 | 0.5436 |
| $R^2$ | 0.9611 | 0.9704 | 0.9867 | 0.9912 | 0.988 | 0.9916 | 0.9869 | 0.9913 |
| Absolute Sum of Squares | 2584 | 1616 | 732.7 | 441.9 | 644.1 | 426.8 | 701.5 | 431.2 |

***Table 3-17.*** *Fitting variables from a monoexponential function applied to the PCr recovery curves displayed in fig 3-45.*

During analyses of post processing approaches, it was realised that the use of a 10-point moving average resulted in a contamination effect (i.e. the averaging included both ischemic and non-ischemic data points at certain time points across the exercise task). This resulted in difficulty establishing the end-exercise PCr signal, at the point of reinstating limb blood flow. Figure 3-46 displays this contamination effect, where using the moving average function across a broad spectra range and continuing to plot details of the exercise task according to the original raw data without accounting for the averaging, results in the PCr appearing to resynthesize prior to reinstating limb blood flow. Thus, averaging over 10 spectra (TR = 4s) results in an excessive standard deviation in signal intensity across this initial post-exercise period, where rapid changes in PCr signal occur.

To circumvent this issue, two alterations were made to the post-processing protocol. Firstly, a 3-point moving average was applied to the post-exercise recovery spectra to enable an improvement in SNR and $^{31}$P peak fitting whilst reducing the standard deviation of $^{31}$P signal intensity across each averaged data point. Additionally, the post-exercise $^{31}$P spectra were separated from the preceding spectra collected during the 16-minute acquisition. The post-processing was then performed in MATLAB on this subset of recovery spectra only. Once the $^{31}$P peaks had been fitted on the post-exercise recovery spectra using the AMARES function in jMRUI, the computed signal amplitudes were normalised to a baseline $^{31}$P spectra generated from a 5-point average obtained under resting conditions at the start of the experimental protocol. Similarly, an end-exercise $^{31}$P spectra was generated from a 5-point average of spectra obtained following contractile failure with ischemia maintained. This same process was performed on a series of 5 end-exercise recovery spectra, collected ~9 minutes after reinstating limb blood flow. The increase in SNR achieved by this averaging under constant physiological conditions enables accurate determination of $^{31}$P metabolite ratios and concentrations (mM) at baseline, end-exercise and end-recovery time-points.

Figure 3-47 displays a PCr recovery curve from an experimental data set as a raw and moving average curve (A & B.) and both curves superimposed (C.) PCr signal amplitude is expressed relative to baseline (calculated from fitting the $^{31}$P peaks to a 5-point average under resting conditions). The end-exercise PCr concentration superimposed on the recovery curve in green (A-C) was calculated by expressing the end-exercise $^{31}$P peak amplitudes relative to baseline. The subsequent exercise recovery data is expressed as a percentage of this baseline signal amplitude.

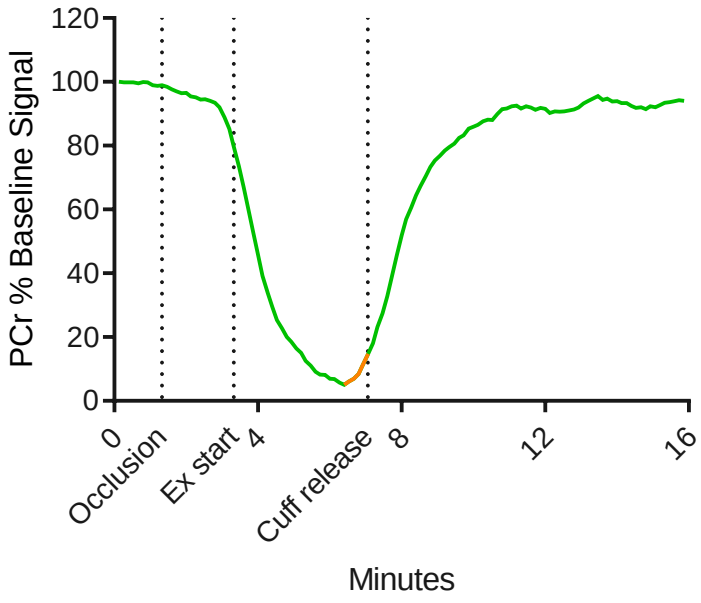



***Figure 3-46.*** *PCr kinetics across the ischemic exercise task. Data presented as a bilateral moving average of ten individual Non-localised $^{31}$P MRS spectra (TR 4s). Key protocol events labelled on x axis. Superimposed orange curve demonstrates "contamination" effect when averaging across $^{31}$P MRS spectra under ischaemic and free flow conditions.*

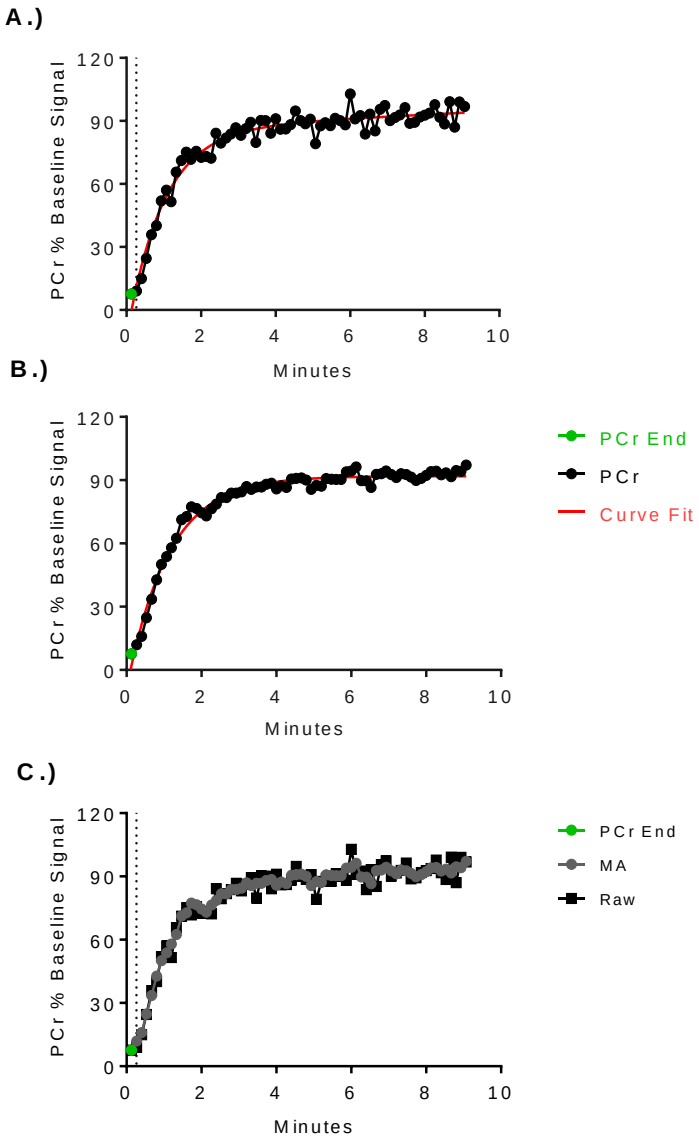



***Figure 3-47****. PCr recovery curves derived from Non-localised pulse acquired $^{31}$P MRS (A.) Fitted from raw spectra TR 8s. (B.) Moving average x3 spectra (C.) raw and moving average recovery curves superimposed. End-exercise PCr concentrations (x5 point average of end exercise spectra) highlighted with green data point. Vertical dashed line highlights point of cuff release.*

## 3.3 Conclusion

The collective work presented in this chapter has established the experimental protocol shown in Figure 3-39, which is applied to healthy volunteers and Crohn's disease patients in Chapter 7.

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# Chapter 4 – Methods & Experimental Protocol

### 4.0 Introduction

This chapter describes the study visits, detailing the experimental protocols and methods employed, an overview of this is provided in Figure 4-1.

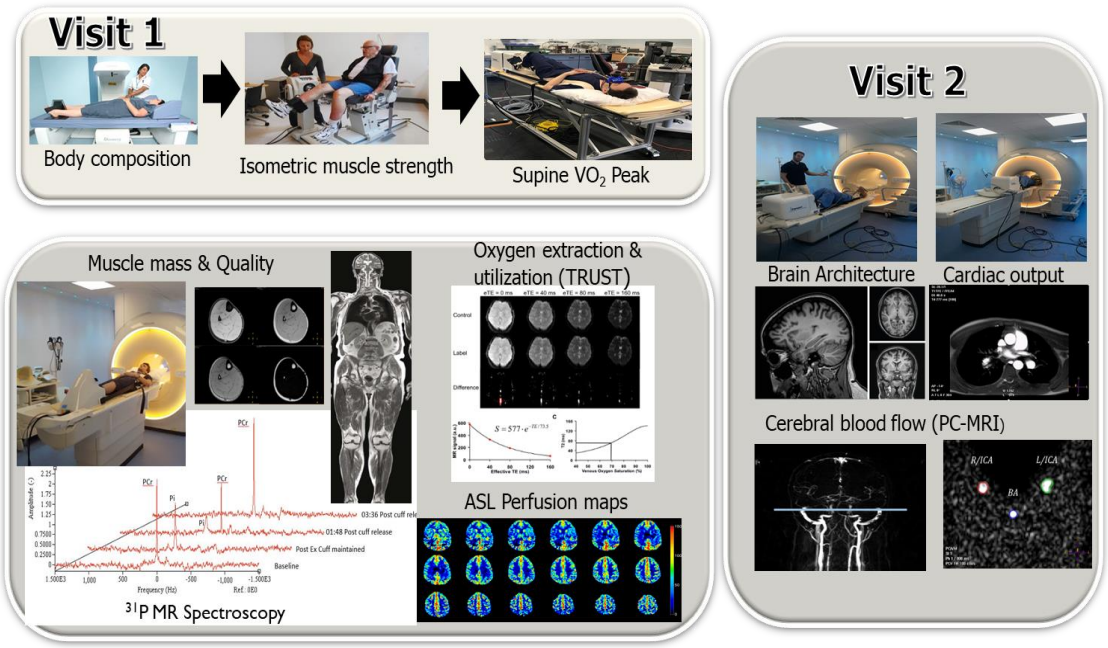


***Figure 4-1.*** *Overview of experimental study visits comprising: Visit 1in the David Greenfield Human Physiology Unit to perform functional phenotyping and Visit 2 at the Sir Peter Mansfield Imaging Centre to collect $^{31}P$ MRS and $^{1}H$ MRI of healthy volunteers and quiescent Crohn's disease patients.*

### 4.1 Ethics Approval

Following completion of the integrated Research Application system application (IRAS, Project ID: 233733), the work contained within this thesis received favourable ethical opinion from the research ethics committee on 22.12.2017 (17/EM/0431) and was approved by the Health Research Authority (HRA) on 10.1.2018. The study conformed to the recognised standards of the Declaration of Helsinki.

### 4.2 Recruitment

Male and female Crohn's Disease (CD) outpatients in remission (aged 16-75 years (BMI <30 $kg/m^2$) were recruited from Nottingham University Hospitals Trust. Disease remission was defined by recent endoscopy or cross-sectional imaging, Harvey Bradshaw index <4, CRP<5mg/dl and faecal calprotectin <50ug/g. Healthy volunteers matched for age, sex and BMI were recruited through advertisement on the Nottingham University Hospitals Trust campuses.

### 4.3 Health screening

Prior to enrolment, all study volunteers underwent routine health screening following written informed consent, (including an electrocardiogram (ECG) full blood count, blood clinical chemistry, liver function test, eGFR), serum for cytokine measures (IL-1, IL-6, TNFα) and Vitamin D. Height, weight and blood pressure was also recorded. Demographic data on: time since diagnosis, ethnicity, education level, employment status, occupation, relationship status, smoking and alcohol history, previous surgery, number of documented relapses needing escalation of treatment in the last 24 months, number of corticosteroids prescriptions in the last 24 months, current use of nutritional supplements, current IBD medication was acquired. Healthy volunteers were screened for pre-existing health conditions that were deemed a

contraindication to study participation. Crohn's disease patients were pre-screened by clinical staff to check they were eligible prior to being invited for health screening. Trait level fatigue perception was estimated by the Multidimensional Fatigue Inventory–20 scale (MFI-20) and the IBD fatigue scale (IBDF scale, Crohn's disease patients only). Mental health status was assessed by the hospital anxiety and depression scale (HADS), cognitive function by the Montreal cognitive assessment (MoCa) and disease activity in patients using the Harvey Bradshaw Index (HBI).

### 4.4 Study Visit One: Functional phenotyping

Volunteers who passed health screening were invited to the MRC/Versus Arthritis Centre for Musculoskeletal Ageing Research laboratory, David Greenfield Human Physiology Unit (DGHPU) at the University of Nottingham Medical School, Queens Medical Centre, Nottingham.

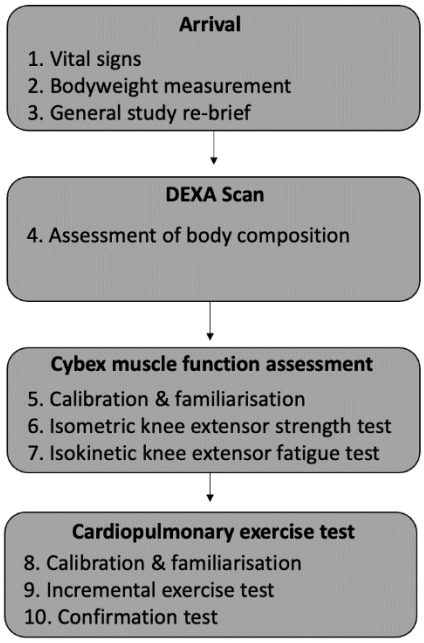


***Figure 4-2*** *Overview of the experimental protocol for laboratory visit.*

Volunteers arrived at 09:00 having maintained their regular dietary pattern and refrained from strenuous exercise for 48 hours prior, and alcohol and caffeine consumption for 24 hours prior. Volunteers were re-briefed on the study protocol, had their resting blood pressure and heart rate measured (DINAMAP, GE healthcare, Chicago, Illinois) and confirmed they wished to proceed with trial participation.

Body composition was assessed via Dual-energy X-ray absorptiometry (DEXA, Lunar prodigy, GE healthcare, Chicago, Illinois) by an IRMER (Ionising Radiation (medical exposure regulations) trained operator to determine bone mineral density (BMD), lean mass (%) and total body fat mass (%).

Volunteers were then taken through a familiarisation and warm-up protocol prior to assessment of knee extensor isometric strength and then work output during repeated isokinetic knee extensions using a Cybex Isokinetic dynamometer (Cybex Norm, Rosemont, Illinois, USA). A calibration procedure was performed, and the volunteer was secured into the ergometer with their knee set at 90˚set up. Volunteers performed ten isokinetic repetitions at 90˚ degrees per second as a warm-up. The knee was then secured at 60˚ and they performed 3 maximal voluntary isometric contractions interspersed with 60 seconds recovery. Volunteers were encouraged to push hard during the three isometric contractions. The knee was then returned to 90˚ and the volunteer again performed five isokinetic warm-up repetitions at 90˚/s. The volunteer then performed a warm-up set of 20 continuous isokinetic knee extensions. Upon completion of the warm-up and familiarisation protocol, volunteers rested for 3 minutes and the set-up of the Cybex dynamometer was re-evaluated to ensure optimal positioning and comfort. It was confirmed that the volunteers understood that the experimental protocol was to be performed under test conditions where both isometric and isokinetic contractions would be performed at maximal effort to the point of volitional exhaustion.

An identical protocol was then re-performed under test conditions. Volunteers performed 3 maximal isometric knee extensions interspersed with 60 seconds recovery. Following this, volunteers then performed 20 maximal isokinetic knee extensions at a constant angular velocity of 90º/s to determine total work output across the task. Each knee extension manoeuvre was initiated from a position of 90 º knee flexion and continued through to the point of full knee extension. After each contraction, the leg was returned passively to the start position (approximately 1.1s) from which the next contraction was immediately initiated. Volunteers were instructed to work as hard as possible on the concentric phase, whilst allowing passive return of the leg to the start position during the eccentric phase. Maximal knee extensor isometric strength was taken as the peak torque value (NM) achieved during the three maximum contractions. Total work done during the isokinetic exercise was taken as the sum of the work values completed during each of the 20 repetitions. Both knee extensor isometric strength and total work done were normalised to leg lean mass established from DEXA scanning.

Following a brief break, volunteers then undertook a familiarisation protocol on a 3T compatible Cardio stepper module (Ergospect GmbH, Innsbruck, Austria). Volunteers were secured into the Cardiostepper by lower limb straps and a waistcoat. The minimal functional resistance was then applied to the air-braked ergometer via the Ergospect software on a PC, whilst the volunteer performed stepping exercise in time with a metronome played at 70 steps per minute. Volunteers performed 3 minutes of low-intensity supine stepping, or until the investigator confirmed that the required power and step frequency was consistently maintained. It was also confirmed that the volunteer was comfortable with the set-up and exercise task. The length of the waistcoat straps was measured and recorded. The knee angle was also measured bilaterally using a goniometer such that the position could be replicated during within-bore exercise during Visit 2. The set-up was optimised such that the volunteer was comfortable with

a knee angle between ~25-30˚. An example of a volunteer on the 3T compatible Ergospect Cardiostepper module is shown in Figure 4-3.

***Figure 4-3**. Example of laboratory set-up for incremental exercise testing using the Ergospect 3T compatible Cardio stepper module with online-expired Gas analysis.*

A heart rate monitor and ECG pads were then placed onto the volunteer and a face mask for online expired gas analysis was fitted. Volunteers then lay in a semi supine position with their hand placed in water at circa 40 ˚C for approximately 10 minutes to dilate superficial veins on the dorsal surface of the hand. A retrograde cannula was then placed into a superficial vein on the dorsal surface of the hand. This hand was immediately placed into a hand-warming unit with the air temperature maintained at 55˚C to arterialise the venous drainage of the hand [2]. Following a 15-minute resting period, a baseline blood sample was taken. All blood samples taken for arterialised-venous blood gas analysis were drawn into heparinised syringes and injected into an i-STAT CG4+ cartridge (~95µL) prior to analyses by a handheld i-STAT blood gas analyser (Abbott Point of Care, USA). Where necessary due to sampling speed of the analyser, samples were capped and maintained on ice prior, to analyses on the I-STAT machine.

Volunteers then performed a continuous, incremental, supine exercise test to determine supine $VO_2$ peak using an on-line expired gas analysis system (COSMED Quark CPET, Rome, Italy). Minute ventilation (VE), carbon dioxide ($CO_2$) production and oxygen ($O_2$) consumption was measured continuously throughout the test. Exercise commenced at a workload of 50W, which was increased at 3-minute intervals. The magnitude of increase was based upon the volunteer's rate of perceived exertion (RPE) recorded during the final 30s of each exercise workload. Using the Ergospect software via a PC, the research team controlled the air-braked ergometer resistance. An arterialised-venous blood gas sample was also taken in the final 30s of exercise at each workload, which was analysed as described above. Volunteers received verbal encouragement and feedback on their power and output and step frequency throughout the test. The test was terminated when the volunteer was unable to maintain the required power output or step frequency consistently for 30s. Upon termination of the test, volunteers rested in a semi-supine position on a bed for 90 minutes prior to a confirmation exercise test. Here, volunteers performed a 3-minute warm-up at a low workload before returning to their previously established peak workload. Exercise was performed to volitional exhaustion using the same termination criteria as the initial test.

### 4.4 Habitual Physical Activity Monitoring

Between experimental visits, the volunteers were given a pedometer device (OMRON HJ-321-E, OMRON Healthcare, Kyoto, Japan) and instructions on how to track their physical activity for a continuous seven-day period prior to their Visit 2 study day. Volunteers manually recorded their daily step-count onto a pre-designed data collection sheet and returned this to the investigator upon arrival at the Visit 2 study day.

### 4.6 Study Visit Two: MRI and $^{31}$P MRS phenotyping

Volunteers arrived for MRI scanning having maintained their regular dietary pattern; they refrained from strenuous exercise for 48 hours prior to the visit. Volunteers were also instructed to avoid alcohol and caffeine consumption for 24 hours prior. A briefing on the study protocol followed, and the MRI health screening and safety questionnaire completed at health screening was confirmed.

#### 4.6.1 MRI Protocol

The MRI protocol were collected on a Philips 3T Ingenia wide bore 70 cm scanner. Prior to within bore exercise protocols, MRI scans were first performed at rest to quantify body composition, brain morphometry and baseline physiology. Volunteers lay in a supine position and were first scanned using a whole body mDIXON protocol with head and abdominal coils to assess whole body muscle mass and fat fraction. Images were obtained six table positions to cover the head to the toes. This also provided the volunteers with a familiarisation to the MRI scanner, prior to within bore exercise.

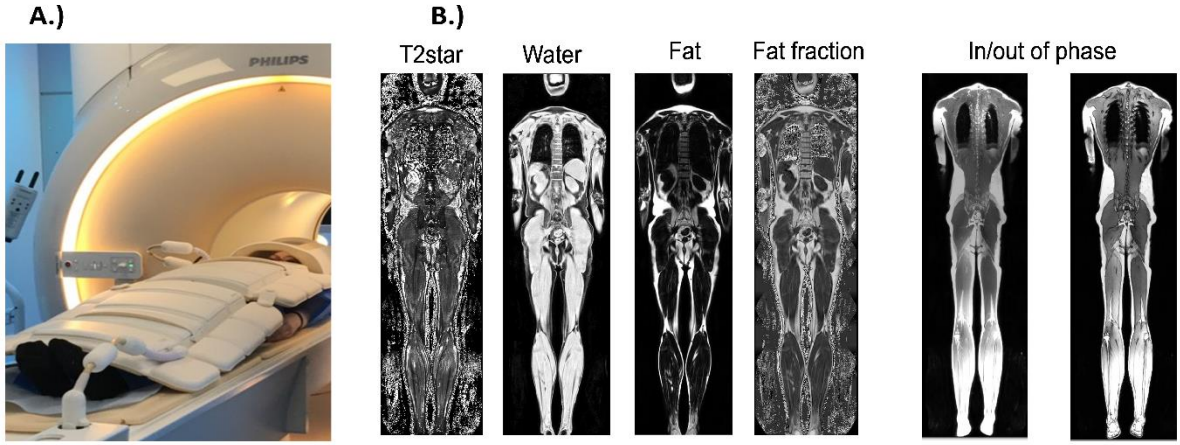


***Figure 4-4***. *A.) Philips 3T Ingenia scanner showing the head coil, together with upper and lower body anterior body coils which together with the integrated FlexCoverage Posterior coils in the scanner bed were used for whole body mDIXON scan acquisition. B.) Example*

Upon completion of the whole body mDIXON protocol, volunteers were given a 15-minute break whilst the 3T compatible Cardiostepper module was set-up and calibrated. Volunteers were then secured into the Cardiostepper module via lower limb straps and the waistcoat. Care was taken to ensure that the knee angle was consistent to that used during incremental exercise testing at the first study visit. This was measured using a goniometer. For all subsequent scans, a Philips Head Neck coil combined with the FlexCoverage Posterior coil, integrated in the table, and base was used to provide good quality head and cardiac images. This also removed the restrictions of using a body coil to image the heart during exercise. This facilitated brain and cardiac scanning at rest and during exercise within the same acquisition period. Heart rate and respiration was continuously measured whilst participants were in the scanner using a PPU device and respiratory belt, and the data exported as Physiological log text files.

***Figure 4-5.*** *Philips HeadNeck coil facilitating the measurement of cerebral blood flow and cardiac output during the Cardiostepper exercise task.*

Volunteers were then scanned under resting conditions to establish cardiac output, global cerebral blood flow, and fractional oxygen extraction fraction at baseline. Volunteers were then instructed to perform supine stepping exercise at a workload equivalent to 50% supine $VO_2$ peak. The air-braked ergometer was controlled externally via a PC in the console room, where power output, step frequency and heart rate were continuously measured. A metronome set to 70 steps per minute was played through the volunteers' headphones. After a 3-minute warm-up period at 50% $VO_2$ peak, the MRI scans collected at baseline were repeated in the same order, such that cardiac output, global cerebral blood flow, and fractional oxygen extraction were quantified during exercise at 50% supine $VO_2$ peak. Upon completion of the within-exercise acquisitions, exercise was immediately terminated and the same sequence of scans performed were repeated whilst the volunteer recovered from exercise.

Upon completion of the exercise recovery scanning, volunteers were given a 30-minute recovery period prior to taking part in the $^{31}P$ MRS protocol. The scan card is outlined in figure 4-6.

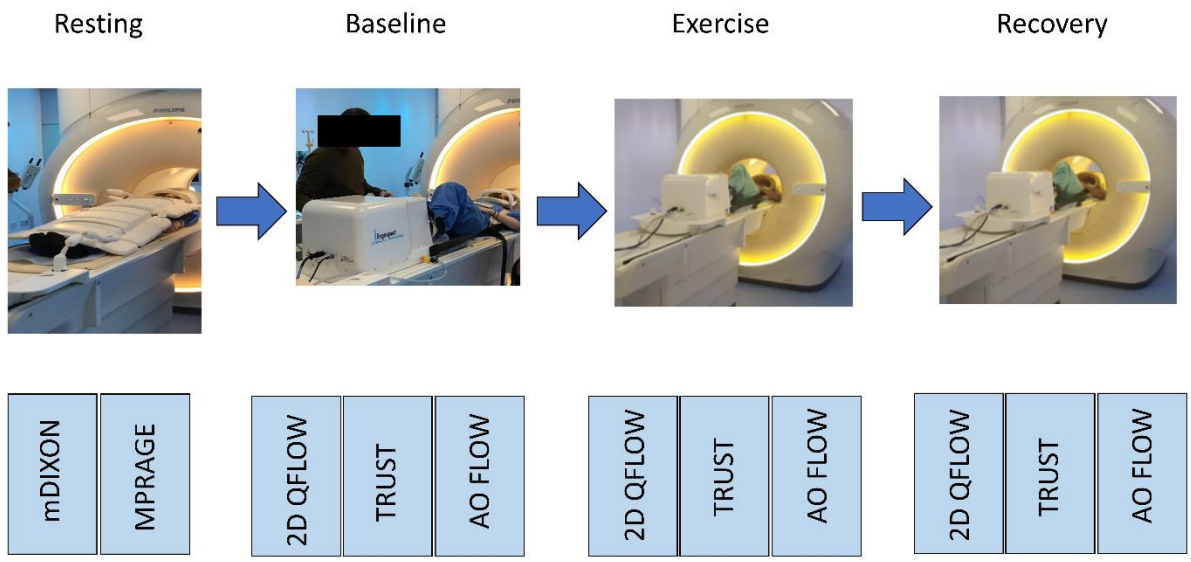


***Figure 4-6:*** *Schematic of MRI scan card showing MPRAGE & mDIXON acqusition under resting conditions followed by 2DQFLOW, TRUST and AO Flow acqusitions across the exercise task.*

### 4.6.2 $^{31}$P MRS Protocol: Assessment of muscle PCr recovery following ischaemic contraction

Volunteers were briefed on the final stage of the experimental protocol. The $^{31}$P MRS protocol was performed on a Philips 3T Achevia scanner with multinuclear capability using the protocol as shown in Figure 5-6. Prior to $^{31}$P MRS, volunteers lay in a feet-first position on the scanner bed with their dominant limb secured into the 3T compatible Trispect module (Ergospect GmbH, Innsbruck, Austria). Maximum calf isometric strength was assessed (see chapter 3). Volunteers were then moved into the scanner. Initially, high resolution mDIXON scans of the calf were acquired in sagittal, coronal and axial planes. A series of three localised $^{31}$P ISIS scans were then acquired across the calf anatomy to establish PCr concentrations in the medial

gastrocnemius, soleus and peronei muscles. Finally, a 16-minute non-localised $^{31}$P acquisition was initiated. Sixty seconds into the acquisition, a member of the research team inside the scan room was instructed to inflate the blood pressure cuff to 250mmHg, and to then signal completion to the research team at the console. After 2-minutes of occlusion to deplete muscle myoglobin [3, 4], the volunteer was instructed to perform repeated plantar flexion exercise at 50% MVC. The air-braked Trispect module (Ergospect GmbH, Innsbruck, Austria) was controlled in the console room by the research team using a PC. The volunteer was verbally encouraged to exercise to volitional exhaustion which typically occurred ~2-3 minutes after ischaemic exercise initiation. Following exercise completion, the cuff pressure was maintained for 30 seconds to necessitate spectral averaging at the end-exercise period. This enabled accurate calculation of end-exercise muscle $^{31}$P metabolite concentrations. The cuff pressure was then released to immediately reinstate limb blood flow. $^{31}$P acquisitions continued during recovery from exercise (~10 minutes). Both the time and dynamics for each experimental event across the protocol was recorded to enable accurate post processing of the spectra.

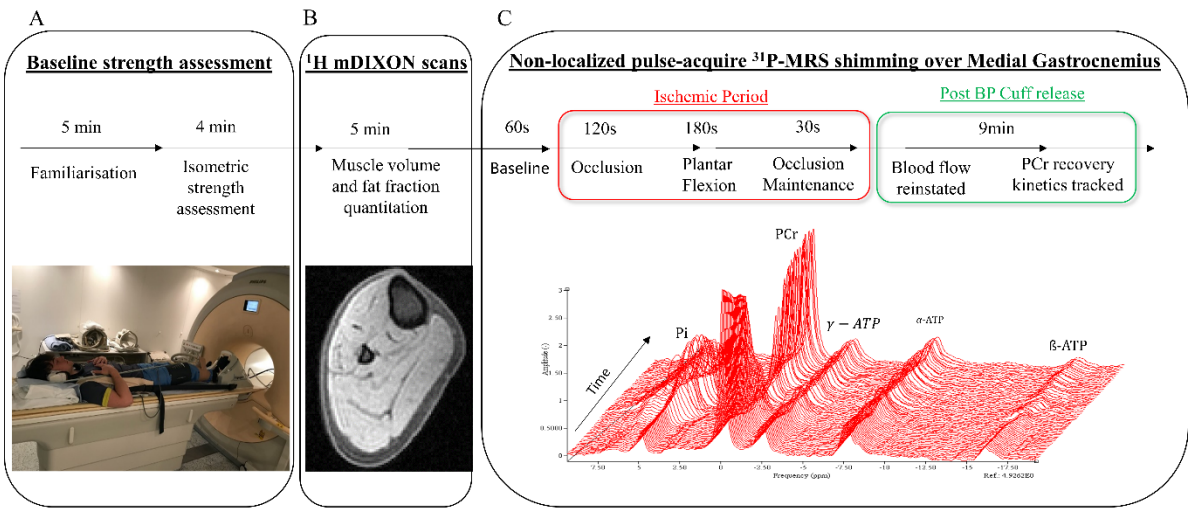


***Figure 4-7.*** *Experimental $^{31}$P MRS protocol encompassing isometric strength assessment, calf composition and PCr recovery kinetics following high-intensity ischemic contraction.*

### 4.6.3 MRI and $^{31}$P MRS Sequences

This section outlines the MRI and $^{31P}$ MRS sequences utilised within the MRI and $^{31}$P MRS experimental protocol.

### 4.6.4 Brain morphometry

A three-dimensional magnetization prepared rapid gradient-echo (3D MPRAGE) is a common sequence utilised for high-resolution brain imaging with $T_1$-weighting. The sequence provides whole brain high spatial resolution images with excellent grey matter/white matter/cerebrospinal fluid (GM/WM/CSF) tissue contrast across in a relatively rapid time-frame, and can be used to compute GM volume, WM volume and CSF volume. A MPRAGE sequence was acquired in the axial-oblique plane along the AC-PC line with 1 mm isotropic resolution; TE/TR = 8.3/3.8 ms, flip angle = 8°, SENSE factor = 2, 160 slices, 256 x 256 matrix. The sequence has wide-ranging clinical application, particularly in disease diagnosis [5] and assessing disease course [6].

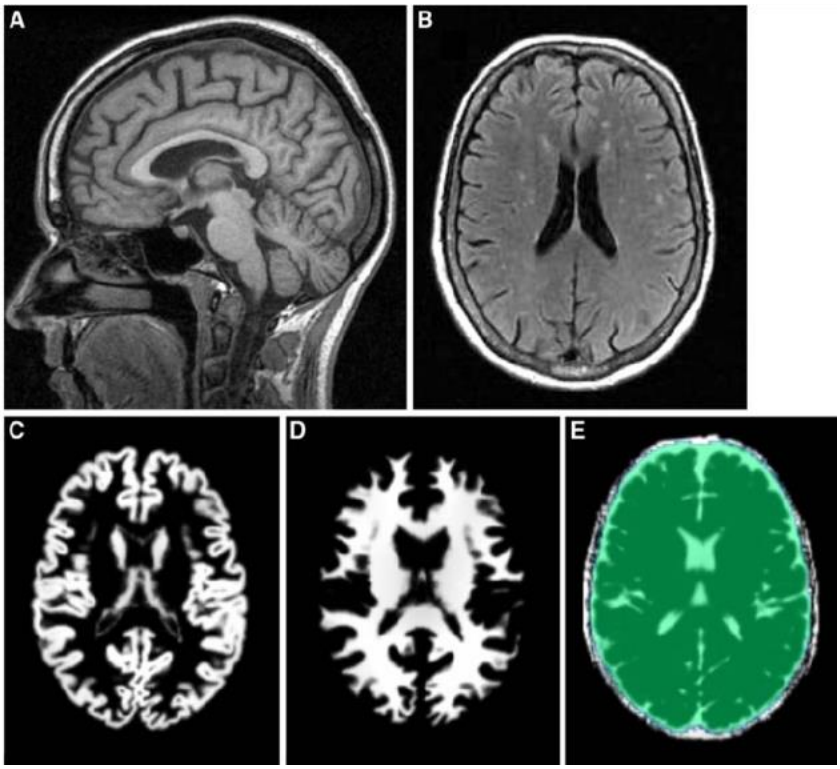


***Figure 4-8**. Example of MPRAGE image and segmentation into GM, WM and CSF. A.) High resolution $T_1$-weighted image in the sagittal view. B) Demonstrates multifocal white matter lesions presenting as hyper intense areas on a FLAIR image. C-E show axial slices of GM, WM and CSF following segmentation and spatial normalisation.*

VBM is an automated analysis method used to delineate brain structural alterations *in-vivo* on a voxel-by-voxel basis by statistical parametric mapping [7]. In VBM the high-resolution $T_1$-weighted MPRAGE image undergoes a series of processing steps including spatial normalisation and segmentation, prior to data smoothing and later statistical parametric mapping [7]. The method allows the delineation of small scale regional differences in GM and WM volumes [8]. In this work, VBM has been used to quantify tissue volumes within the brain, allowing HV and CD group comparisons. Of relevance in VBM analysis, is the confounding factor of head size. To circumvent this issue, the volumes of each tissue type for an individual are normalised to the total intracranial volume (TIV).

In the literature, VBM has been widely utilised to assess brain structural changes associated with ageing [9], psychological illness [10] and of relevance to the work in this thesis, chronic

fatigue syndrome (CFS) [11, 12]. An outline of the processing methods used during the VBM analyses of structural data within Chapter 8 is detailed below.

### 4.6.5 Central haemodynamics and brain fractional oxygen extraction

Phase-contrast MRI (PC-MRI)

PC-MRI is used to measure blood flow, the basic premise relates to measuring the net phase shift of moving spins within arterial blood during the application of a bipolar magnetic field gradient, with the phase shift being directly proportional to their velocity. PC-MRI acquires two data sets, with flow sensitivity varied between these acquisitions, and controlled by the strength of an applied bipolar gradient pair of equal magnitude and opposite polarity. The first data set is acquired without flow sensitivity while the second is acquired with a flow sensitive sequence of a defined velocity encoding, $V_{ENC}$. Stationary tissue spins will therefore experience no phase shift, while moving blood spins experience a non-zero phase shift. The two data sets are subtracted, producing a PC image. Since the phase shift is proportional to the spin's velocity, quantitative assessment of flow velocity is achieved. This is illustrated in Figure 5-9.

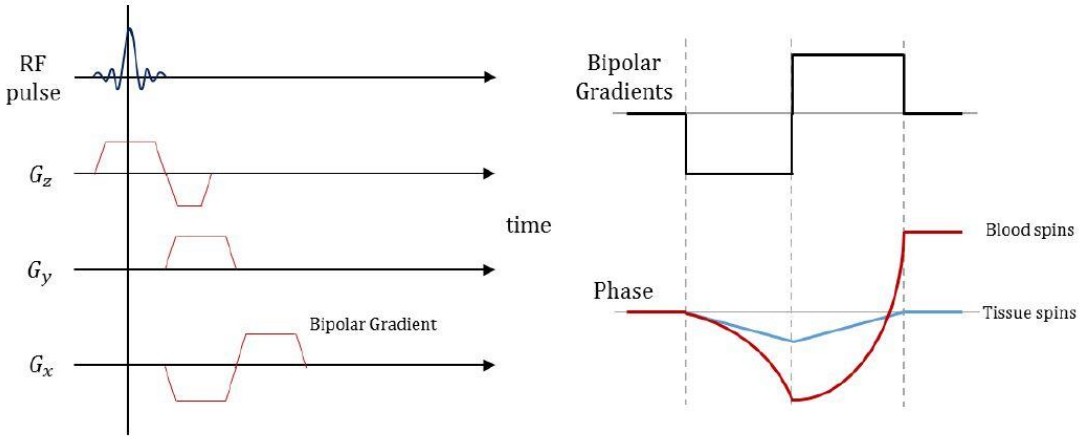


***Figure 4-9**. Pulse sequence diagram for PC-MRI demonstrating the effect of bipolar gradient application on the phase of spins. This is shown for stationary tissue spins and blood moving through a vessel.*

PC-MRI has become a popular method for non-invasive quantitation of both cerebral haemodynamics to assess blood flow through the internal carotids and basilar artery [13] and also cardiac output by measuring blood flow in the ascending aorta (AO) [14]. Importantly, this provides a measure that is independent of exogenous tracer use and so can be used for repeated measures.

In this work, blood flow in the left and right internal carotid arteries as well as the basilar artery were assessed using cardiac triggered PC-MRI. Slices were positioned perpendicular to the direction of flow in the vessel of interest. Scan parameters were FOV = 280 x 77mm2, spatial resolution = 0.75 x 0.75mm2, slice thickness 6mm, SENSE factor 3, TE/TR = 6.5/15ms, FA = 25°, VENC 100cm/s, NSA = 2. The sequence was cardiac triggered using the PPU with the data acquired at 30 time points across the cardiac cycle. The duration of the scan was thus

dependant on the subject's heart rate.

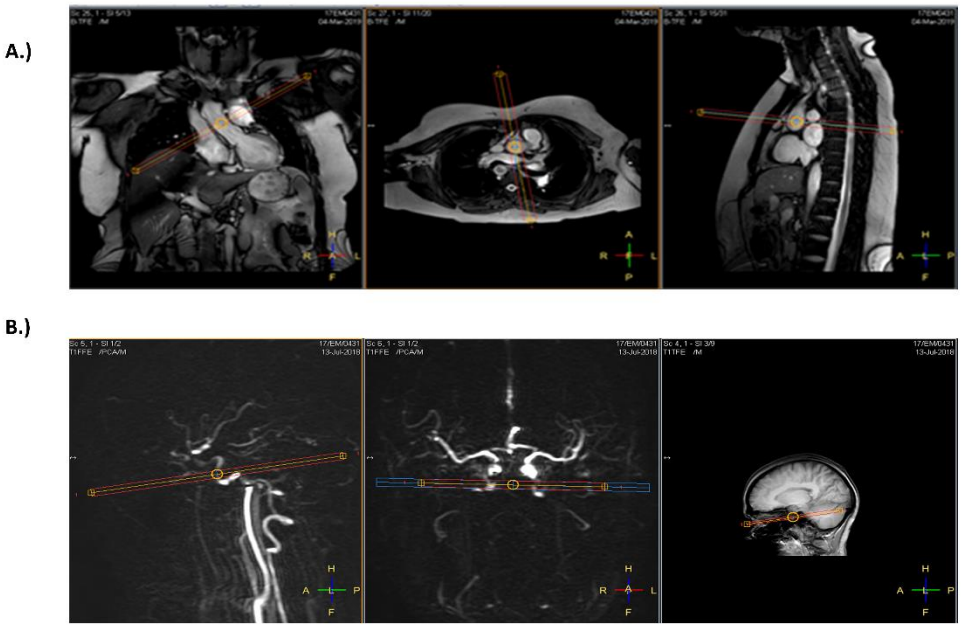


***Figure 4-10*** *Planning scans for phase contrast MRI sequences A) Ascending Aorta (AO) flow B) Internal carotid flow.*

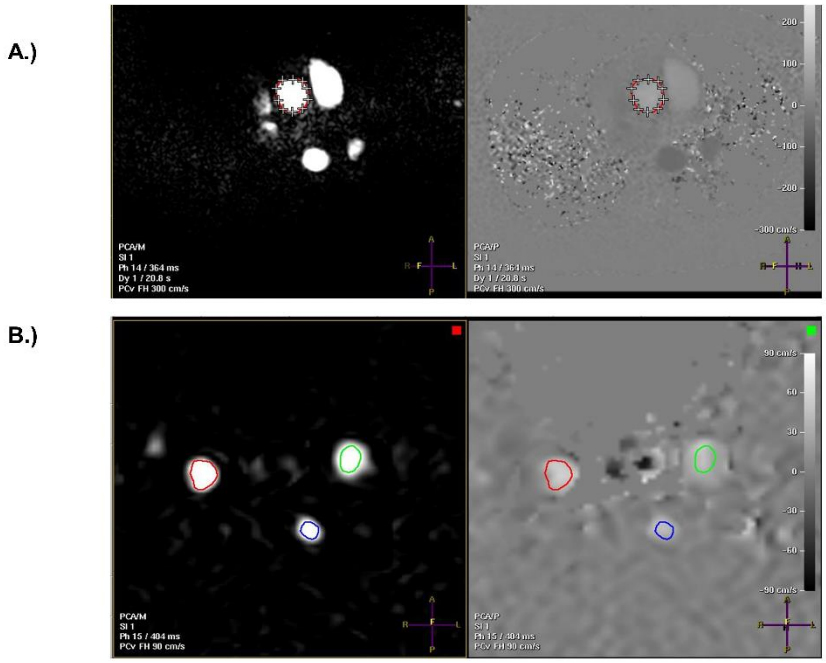


***Figure 4-11****. Example PC-MRI analyses in Philips View forum. A.) AO flow analyses and B.) 2D Qflow analyses. ROI's drawn around target vessel (ascending aorta for AO flow and internal carotid arteries and basilar for 2D Qflow) on magnitude image shown on the left. Corresponding phase image shown on the right, where ROI can be adjusted.*

Aortic flow was acquired using a free breathing PC-MRI measure with scan parameters of FOV = 280 x 77mm2, spatial resolution = 0.75 x 0.75mm2, slice thickness 6mm, SENSE factor 3, TE/TR = 6.5/15ms, FA = 25°, VENC 100cm/s, NSA = 2.

Details of the post processing methods used for the PC-MRI sequences used within this study to quantify cerebral blood flow and cardiac output are described below.

### 4.6.6 $T_2$-Relaxation-Under-Spin-Tagging (TRUST-MRI)

TRUST-MRI is a more recently developed method for quantitative measurement of cerebral metabolism [15]. Application of the TRUST method to the brain utilises the spin labelling principle to isolate blood signal in the sagittal sinus [16]. Using flow-insensitive $T_2$-preparation pulses, the $T_2$ relaxation time of the blood can be found and converted into blood oxygenation using a calibration plot [17] which enables quantitation of venous oxygenation, $Y_v$ and the oxygen extraction fraction percentage. Scan parameters for the TRUST sequence (Figure 5-11) used had an in-plane field of view (FOV) of 230 mm x 230 mm, spatial resolution 3.44 x 3.44 mm2, slice thickness 5 mm, TI = 1022 ms, and TR = 3000 ms per label/control pair. Four eTEs were collected at 1, 40, 80, 160 ms corresponding to 0, 4, 8 and 16 refocussing pulses in the $T_2$ preparation with an interval $_{cpmg}$ = 10 ms (Carr-Purcell-Meiboom-Gill). The scheme comprised a flow alternating inversion recovery (FAIR) labelling scheme with selective/nonselective thickness of 25/300 mm.

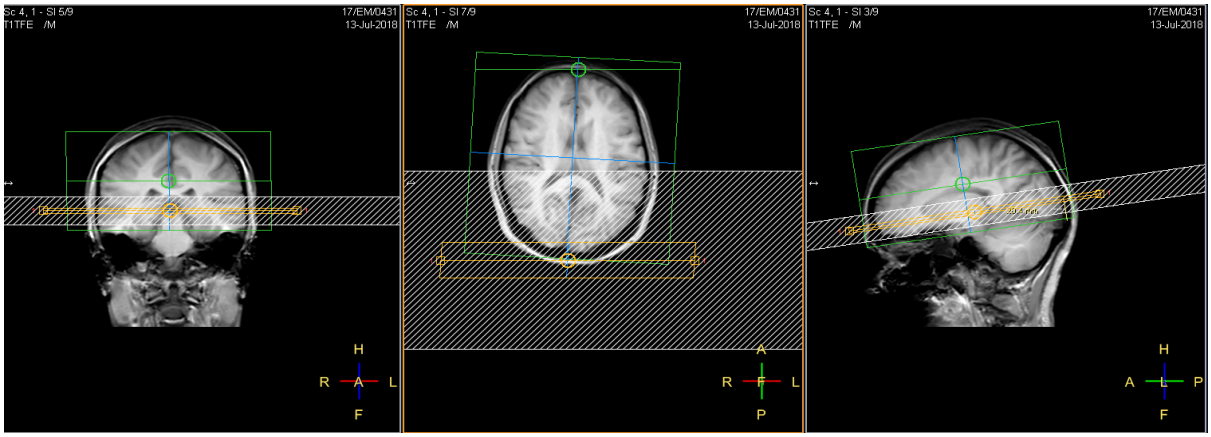


***Figure 4-12.*** *TRUST sequence planning. The yellow box shows the imaging plane cutting through the sagittal sinus. The white box shows the FAIR selective slab, and the green box shows the shim box.*

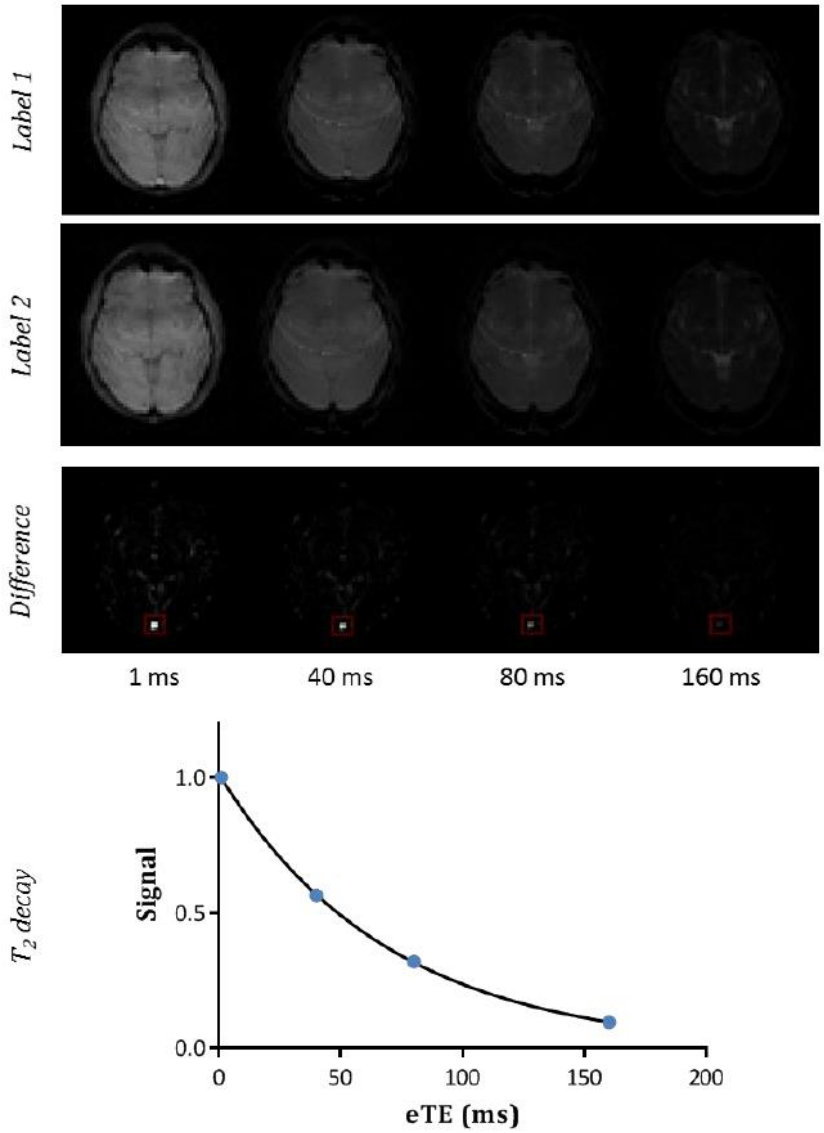


***Figure 4-13.*** *TRUST analysis schematic. Label and control images are acquired at 4 TEs of 1, 40, 80 and 160 ms. A difference image is created by the pairwise subtraction of the label and control data at each TE to isolate signal in the sagittal sinus. Four voxels in the sagittal sinus with the highest intensity (highlighted in red) are interrogated to the fit the $T_2$ decay of the venous blood.*

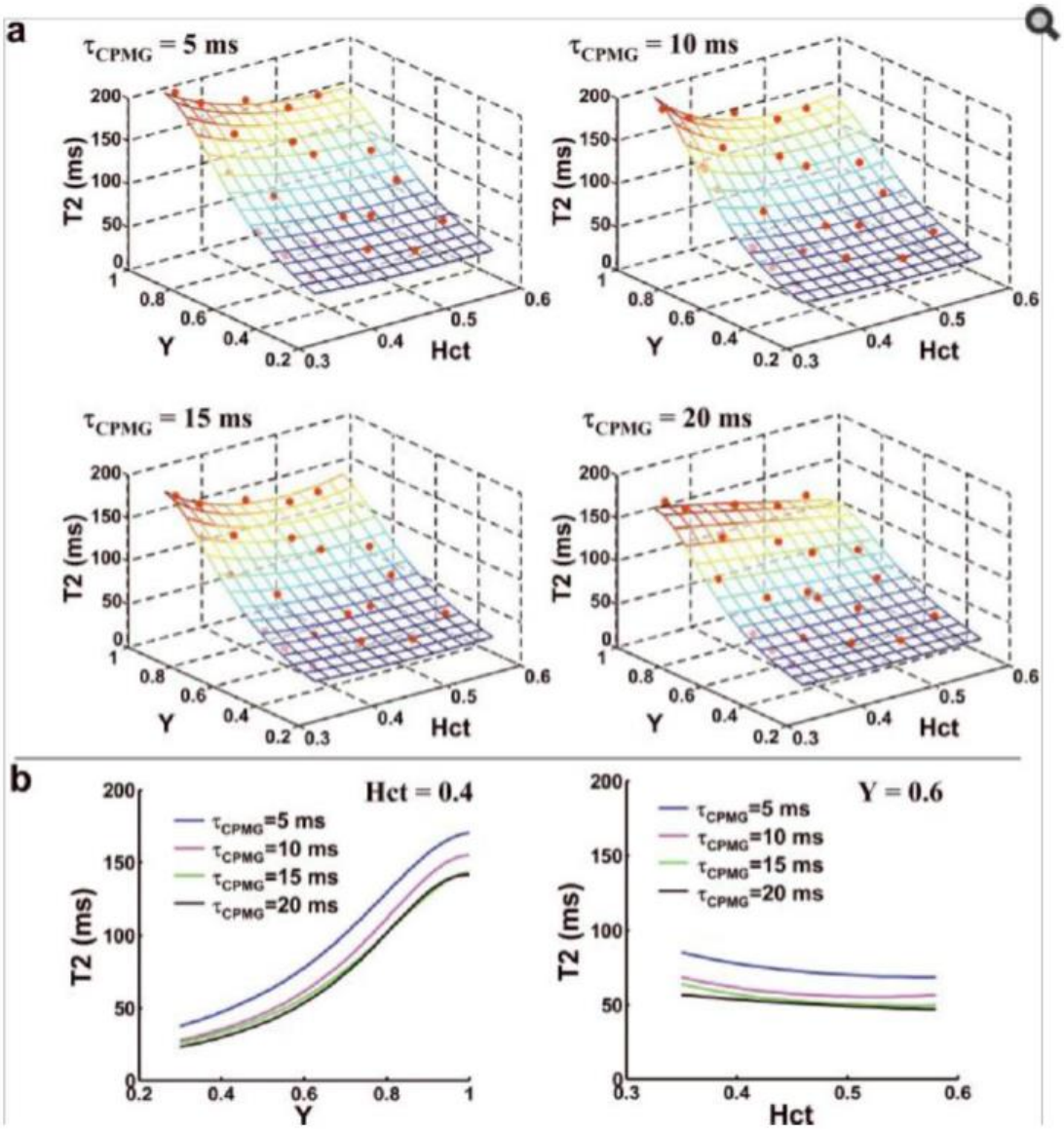


***Figure 4-14.*** *TRUST calibration plots are used to convert the venous blood $T_2$ relaxation time into venous oxygenation, Yv.* [1]

### 4.6.7 mDIXON

The Dixon technique exploits the fact that water and fat molecules precess at different rates. As such, over time, they will alternate between being in-phase and opposed-phase. Acquiring both in-phase and opposed-phase images simultaneously allows the images to be combined in result in a total of four sequences:

in-phase image = (water + fat)

opposed-phase image = (water - fat)

fat only image = in-phase - opposed phase = (water + fat) - (water - fat)

water only image = in-phase + opposed phase = (water + fat) + (water - fat)

Example images are shown in Figure 5-4, B. By collecting data at a range of echo times in a mDIXON scheme it is also possible to fit for $T_2$* and fat fraction.

## 4.7 Leg $^{1}$H MRI and $^{31}$P Magnetic Resonance Spectroscopy Sequences

The following sequences were collected on the 3T Achieva scanner for imaging of the leg.

### 4.7.1 mDIXON images

A series of high-resolution DIXON images (in-phase and opposed phase) were acquired in the transverse, sagittal and coronal planes to establish correct positioning of the limb and to enable shimming of the region of interest (ROI). Image parameters were as follows (FOV 208 x 189 x 250 , matrix size 140 x 126, 50 slices, flip angle 10˚, TR/TE1/TE2 3.4/1.1/2.2).

### 4.7.2 $^{31}$P MR- Spectra

$^{31}$P spectra were collected both non-localized and localized using a 14 cm $^{31}$P surface coil (Philips Medical Systems, Best, Netherlands). Non-localized pulse-acquire $^{31}$P-MR spectra were obtained using a TR 4 s, TE 0.10ms, spectral bandwith 3000 Hz, samples 4096. $^{31}$P-MR Image Selected In Vivo Spectroscopy (ISIS) data was acquired using a TR 10 s, TE 0.10ms spectral bandwidth 2000 Hz, VOI 30x10x100 mm, 4096 samples.

### 4.8 Post processing of MRI and $^{31}$P MRS Data

Please see chapter 3 for a detailed overview of $^{31}$P MRS post processing and data analysis.

#### 4.8.1 Voxel-based morphometry Brain Analysis

Voxel-based morphometry (VBM) was used to assess GMV, WMV and CSF volume, and surface-based analysis (SBA) was used to assess cortical thickness (CT). Pre-processing for both analyses was conducted in CAT12 (Computational Anatomy Toolbox) (version 12.6 r1450; http://www.neuro.uni-jena.de/cat/) within SPM12 (version 7771; http://www.fil.ion.ucl.ac.uk/spm/softw are/spm12/) using MATLAB version 9.7 (R2019b, MathWorks). This included bias-field and noise removal, skull stripping, segmentation into grey and white matter, and normalization to MNI space using DARTEL to a 1.5 mm isotropic adult template provided by the CAT12 toolbox. Intensity modulation of the normalized tissue segments accounted for both global affine transformations and local warping. Image data quality was assessed in all individual images from basic image properties, noise and geometric distortions (e.g. due to motion) with an image quality rating (IQR) of 84% (1.5% SD), representing good image quality. Grey and white matter segments were spatially smoothed using a 12 mm FWHM Gaussian smoothing kernel, and edge effects between tissue types avoided by excluding all voxels with values < 0.1 (absolute threshold masking). Results of global volume measures are described in Chapter 6.

For CT analysis, $T_1$-weighted images underwent tissue segmentation to estimate white matter distance, i.e. projection-based thickness, which handles partial volume information, sulcal blurring, and asymmetries, and topological correction performed based on a spherical harmonics approach. Spherical registration was used to adapt the volume-based diffeomorphic DARTEL algorithm to the cortical surface. Finally, to increase processing speed, all scans were

resampled to a higher-resolution 164,000 mesh compatible with FreeSurfer data, followed by smoothing with a gaussian kernel of 15 mm FWHM.

## 4.9 Central vascular and metabolic responses to exercise

### 4.9.1 Phase contrast MRI analysis

PC-MRI data were processed using View forum software (Philips Medical Systems, Netherlands). An ROI was drawn around the walls of the internal carotid arteries and the basilar artery using the magnitude image. The ROI's were then propagated across all phases in the cardiac cycle and manually checked and edited to optimise fitting. The magnitude image was cross-referenced with the phase data to identify any aliasing. This enabled calculation of blood velocity (mm/s) and vessel cross sectional area ($mm^2$). The product of velocity and area within the ROI represents flux in each vessel. Flux values across the internal carotid arteries and the basilar artery were then combined across vessels to compute global cerebral blood flow. The cerebral blood flow value was presented as both absolute values and normalised to grey matter volume to result in gmCBF, and thus account for structural atrophy.

AO flow data were analysed using the same approach. An ROI was drawn around the ascending aorta using the magnitude image, which was propagated across the cardiac cycle. The computed heart rate and stroke volume was used to calculate cardiac output. This was represented both as absolute values and aortic cardiac index, after normalising to body surface area.

### 4.9.2 Cerebral oxygen metabolism – TRUST analysis

TRUST data were analysed using in-house MATLAB code according to previously described methods [15]. Venous blood signal in the superior sagittal sinus (SSS) was computed from the

pairwise subtraction of label and control images to obtain difference images for each eTE. In the resultant difference images, an ROI of four voxels with the highest difference signal created a mask for spatial averaging of the signal. Blood $T_2$ relaxation was then calculated within the SSS ROI by fitting to the mono-exponential signal decay. The blood $T_2$ relaxation time in the SSS was then converted into venous oxygenation, Yv, using the calibration plot [1] as shown in Figure 4-13.

Arterial oxygenation, $Y_a$ was calculated as described by Peng et al [18]

$$Y_a = 99.77 - 0.036 \times age - 1.235 \times sex + 0.021 \times age \times sex \quad (2)$$

where *age* is in years and *sex* is defined as 0 for females and 1 for males [19].

Oxygen extraction fraction was calculated by

$$OEF = Y_a - Y_v \quad (3)$$

gCMRO2 was calculated as described by Liu et al [20]

$$gCMRO_2 = gCBF \times (Y_a - Y_v) \times C_h \quad (4)$$

where *gCMRO2* and *gCBF* are global cerebral metabolic rate of oxygen and global cerebral blood flow (gCBF) and $C_h$ is the capacity of blood to transport oxygen. $C_h$ values were calculated for each subject by converting their individual haemoglobin concentrations obtained during routine blood testing (g/dL) to (mmol/L). When haematocrit data was unavailable, a literature value was assumed (8.34 mmol/L or ~13.4g/dL Hemoglobin). $C_h$ was then adjusted for age assuming a decline rate of 0.0079 mmol/L per year [21].

gmCMRO$_2$ was then corrected for brain atrophy to account for any disease or age related loss of grey and white matter between groups [18].

$$\text{gmCMRO}_2 = \frac{tCMRO_2}{V_{gm}+rV_{wm}} \times \frac{1}{\rho} \quad (5)$$

where *gmCMRO$_2$* is the *CMRO$_2$* normalised to grey matter volume, $V_{gm}$ and $V_{wm}$ are the grey and white matter volumes, r is the ratio between white and grey matter volume and ρ is a constant relating to the mass density of tissue, which is assumed to be 1.06 g/ml [22]

### 4.9.3 High–resolution DIXON images to assess calf anatomy

To obtain muscle volume and fat fraction within the muscle from the DIXON images, an FSL and MATLAB pipeline was used. First the water image was fed into FSL's FAST function, which was set to segment three tissue types. The image was then cleaned by removing the skin on the perimeter of the image, using a Euclidian distance transform for extraneous objects, and using the fat fraction map to eliminate any instances where the fat fraction was above 60%. From this the muscle volume was then estimated and the mean fat fraction within this computed.

### 4.9.4 $^{31}$P MRS

$^{31}$P spectra were analysed using jMRUI Beta 6.0,$^{31}$P-MR spectra were apodized to 10Hz with Lorentzian fitting. $^{31}$P spectra peaks including inorganic phosphate (Pi) PCr, and ATP subunits $(\gamma - ATP, \alpha - ATP, ß - ATP)$ were fit using the AMARES function with prior knowledge. The exercise kinetics for PCr was expressed relative to baseline signal amplitude. Cytosolic pH was calculated using the chemical shift difference $(\delta)$ between Pi and PCr peaks:

$$pH = pK + log\left(\frac{\delta 1-\delta 0}{\delta 0-\delta 2}\right) \quad (6)$$

where p$K$ = 6.75, $\delta 1$ = 3.27, $\delta 2$ = 5.63.

Free ADP was estimated as:

$$[\text{ADP}] = \left(\frac{[TCr]}{[PCr]} - 1\right)\left(\frac{[ATP]}{(K[H+])}\right) \quad (7)$$

where $K$ = 1.66 *$10^9$ l mol$^{-1}$ and ATP and TCr are assumed to be 8.2 mM and 42.5 mM/ L cell water.

The PCr recovery curve was fit to a mono-exponential function in GraphPad prism:

$$PCr(t) = PCr_{initial} + (PCr_{end} - PCr_{initial})(1 - e^{-kt}) \quad (8)$$

where $t$ is the time from the start of recovery, $PCr_{initial}$ and $PCr_{end}$ is the PCr content at the initial and end of recovery phases.

The initial rate of PCr resynthesis was estimated as:

$$V_{PCr} = k_{PCr} \times \Delta[PCr] \quad (9)$$

where $k_{PCr}$ is the rate constant of PCr resynthesis and $\Delta[PCr]$ is the end recovery PCr concentration minus the end-exercise PCr concentration in mM/L cell water.

$Q_{max}$ was estimated as:

$$Q_{max} = k_{PCr[basal\ PCr]} \quad (10)$$

where $k_{PCr}$ is the rate constant of PCr resynthesis and basal PCr is the resting PCr concentration in mM/L cell water.

$Q_{max\ ADP}$ was estimated as:

$$Q_{max} = V_{PCr}\left(1 + \frac{Km}{[ADP]end}\right) \quad (11)$$

where [ADP]*end* is the ADP at the end of exercise, $V_{PCr}$ is the initial rate of PCr resynthesis and $K_m$ = 30μm/L is the equilibrium constant of the creatine kinase reaction.

### 4.10 Conclusion

This chapter has outlined the exercise laboratory, MRI and $^{31}$P MRS measurements used in this thesis. The results will be described in Chapters 5-7. A consort diagram displaying volunteer recruitment and progression through the study is displayed in figure 4-15.

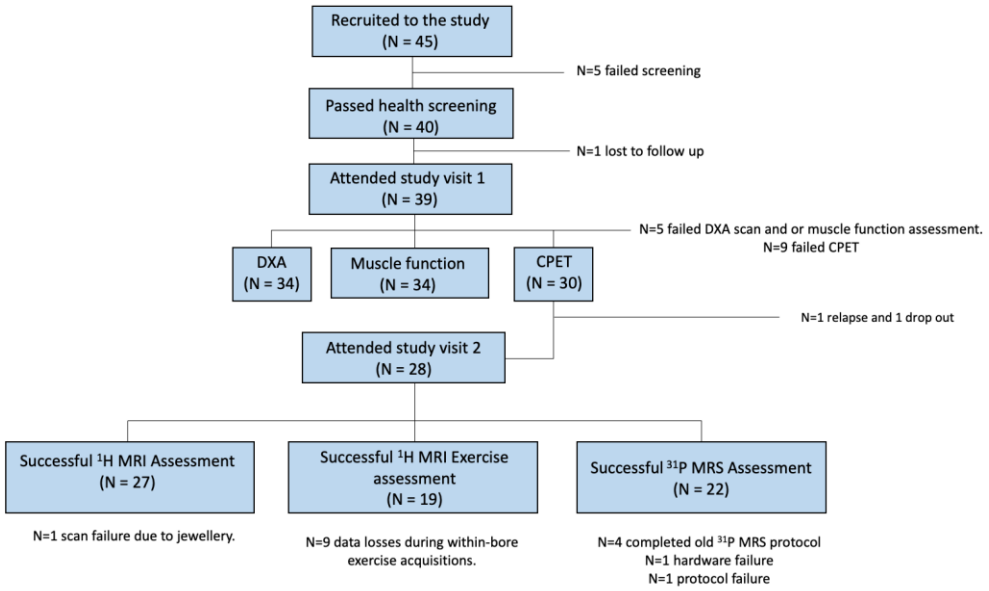


***Figure 4-15:*** *Consort diagram displaying recruitment of volunteers and progression through the study. Stratification of volunteers (HV & CD) and details of successful data acquisition from MRI measurements is covered in chapter 6 and 7.*

## 4.11 References


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# Chapter 5. Body composition, peripheral muscle strength and fatiguability in quiescent Crohn's disease.

**Abstract**

IBD patients commonly present with increased fatigue perception secondary to peripheral muscle weakness, accelerated loss of muscle force and reduced cardiorespiratory fitness relative to healthy control volunteers.

The aim of the experimental work within this chapter was to assess whether quiescent Crohn's disease patients report an increased perception of fatigue relative to age and BMI matched healthy volunteers, and to compare body composition, peripheral muscle weakness and premature fatiguability between these two cohorts.

Crohn's disease patients and healthy volunteers were matched for age and BMI. Volunteers underwent DEXA scanning to establish body composition and bone mineral density. Habitual physical activity was measured over seven days using a pedometer. Maximal isometric knee extensor strength and total isokinetic knee extensor work was assessed by isokinetic dynamometry.

The mean fatigue perception scores on the general domain of the MFI-20 scale were increased in Crohn's disease patients relative to healthy volunteers ($P = 0.001$) although physical domain fatigue scores were no different between groups. Maximal isometric knee extensor strength and total isokinetic work during 20 maximal repeated isokinetic contractions were no different in Crohn's disease patients compared to healthy volunteers. Body composition was also no

different between the two groups. Habitual physical activity was less in Crohn's disease patients than in healthy volunteers *(P* = 0.03).

Quiescent Crohn's Disease patients self-reported increased fatigue perception on the general domain of the MFI-20 scale, but not in the Physical domain. Crohn's Disease patients also performed less habitual physical activity than healthy volunteers. However, there was no evidence of peripheral muscle weakness or premature fatiguability in the Crohn's disease patients compared to healthy volunteers.

## 5.1 Introduction

The prevalence of an increased perception of fatigue in IBD [1] and accompanying premature exercise fatigue [2] was highlighted in chapter 3.

IBD fatigue symptomology is particularly pervasive in that it tends to persist in a large proportion of patients who achieve clinical remission [3].This appears to be independent of known reversible causes. A number of disease related factors associate with fatigue such as disease activity [4], inflammation, medications [5], biochemical abnormalities [1], nutrient deficiencies and metabolic disorders such as sarcopenia [6] and obesity [7]. Although the absence of mechanistic data pertaining to IBD fatigue means that the aetiology remains poorly understood [8, 9] and treatment options are sparse.

Peripheral muscle weakness is reported in IBD cohorts [10-12]. Furthermore, patients with an increased perception of fatigue appear to be weaker (i.e. reduced peripheral muscle strength) relative to patients with a lower perception of fatigue [2]. Whilst these data implicate peripheral muscle weakness in IBD fatigue aetiology, there is also evidence that maximal isometric knee extensor strength is maintained in IBD patients with an increased perception of fatigue, relative to healthy controls [13]. Furthermore, previous assessment of knee extensor muscle

fatiguability in IBD has been performed using experimental protocols with a high reliance on central drive and skeletal muscle mass. For example, van langenberg *et al* assessed knee extensor muscle fatiguability via repeated maximal effort isometric contractions [13]. Similarly, Wiroth and colleagues had Crohn's disease patients and healthy controls perform a bilateral, maximal isometric knee extension task for 15s to quantify lower limb endurance [10]. These protocols used to assess muscle fatiguability are of questionable relevance. The high reliance on the rapid force generating capacity of the muscle is essentially measuring peripheral muscle strength capabilities as appose to the ability of skeletal muscle to maintain the required force output during exercise [14]. This limits the validity of these data.

Peripheral muscle strength correlates with muscle mass [15], which is in line with the correlation between DEXA estimated lean body mass and thigh strength in quiescent IBD patients and healthy control's [16]. This suggests that the peripheral muscle weakness reported in IBD [2, 10-12] may be attributable to muscle atrophy. The prevalence of IBD sarcopenia [17] and obesity [7, 18] is well recognised. Schneider *et al* reported a maintenance of total body lean mass (43.8 ± 9.4 vs 46.7 ± 10.1 kg) and fat mass (16.2 ± 8.9 vs 16.1 ± 4.9 kg) values estimated by DEXA scanning in a cohort of 82 Crohn's disease patients relative to 50 healthy controls. Although there was a loss of appendicular lean mass in the Crohn's disease patients (6.0 ± 1.1 vs 6.5 ± 1.2, $P < 0.05$). Earlier data in 32 quiescent patients with long standing Crohn's disease showed that total body lean mass (48.6 ± 9.3 vs 49.7 ± 11.6 kg) and fat mass (17.6 ± 7.9 vs 19.7 ± 6.3 kg) was unchanged relative to 32 healthy control subjects [16]. A more recent systematic review of body composition in IBD reported less fat free mass (FFM) in 28% of IBD patients across 6 studies of 174 patients relative to controls, whereas FFM was unchanged in 65% of patients across 11 studies (N=394) relative to healthy controls. Similarly, fat mass appears to be largely maintained in Crohn's disease, as 66% of patients across 9 studies (N=419 subjects) had comparable fat mass to healthy controls, whilst 31% had reduced fat

mass and only 3% had greater fat mass than control volunteers [19]. In contrast, bone density is consistently reduced in IBD. A comparison of 241 CD patients' vs 210 healthy controls revealed a statistically significant reduction in bone density in 87% of Crohn's disease patients relative to healthy controls [19].

### 5.2 Aims

As part of our phenotyping experiments in IBD patients, we sought to assess self-reported fatigue perception, body composition, maximum isometric thigh strength, isokinetic thigh fatiguability and habitual physical activity levels in quiescent Crohn's disease patients, relative to age, gender and BMI matched healthy controls. Based on previous data it was hypothesised that the Crohn's disease patients present with increased fatigue perception, less lean body mass and increased adiposity, secondary to thigh weakness and premature thigh fatiguability and less habitual physical activity.

### 5.3 Methods

The following experimental protocol and methods are described in detail in chapter 4. Briefly, following a routine health screening visit, study volunteers completed visit one at the David Greenfield Human Physiology Unit (DGHPU, University of Nottingham)as previously described. Fatigue perception was estimated scale in the healthy volunteers and Crohn's Disease patients using the General and Physical domains of the MFI-20 and using IBD Fatigue scale scores (Crohn's Disease patients only). Body composition was estimated by DEXA scan and muscle function (maximal isometric thigh strength and fatiguability during repeated maximal isokinetic contractions) was assessed using a Cybex isokinetic dynamometer.

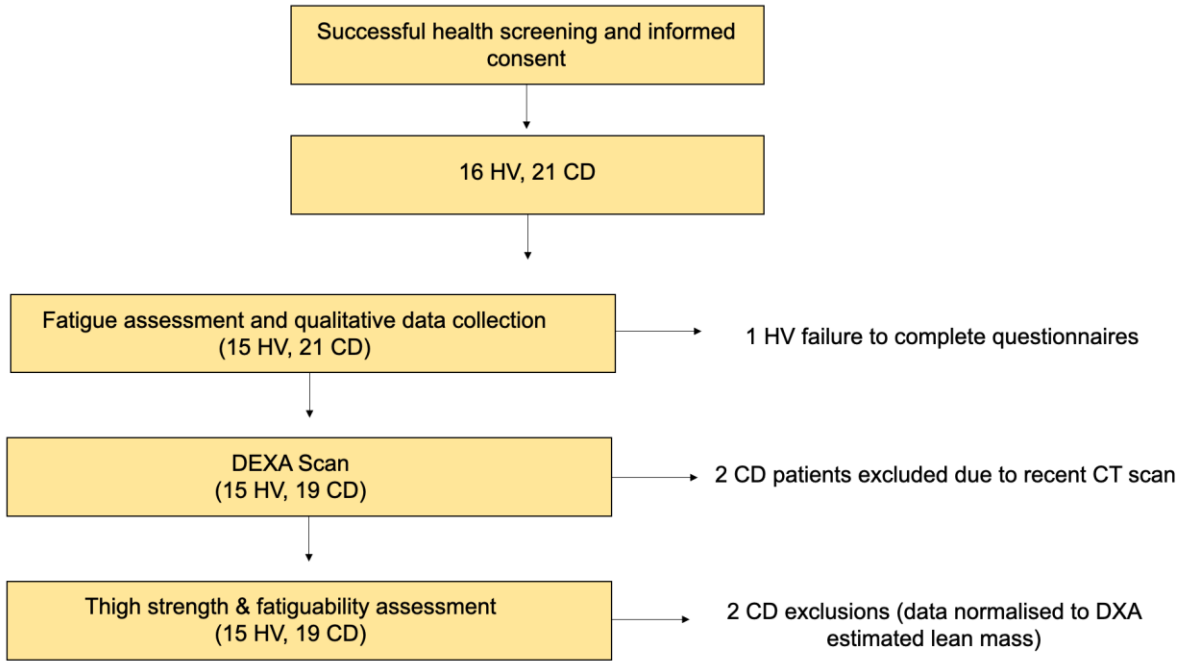


***Figure 5-1.*** *Consort diagram detailing volunteer progression from health screening through to the laboratory visit.*

### 5.3.1 Body composition

Volunteers underwent DEXA scanning to establish body composition and bone mineral density in the two experimental groups. Lean mass index (total body lean mass /ht$^2$) and appendicular lean mass index (appendicular lean mass/ht$^2$) [20] was then calculated. Muscle strength and fatiguability data were normalised to leg lean tissue mass (i.e. MVC in NM/ kg and total work Nm / kg) to ascertain whether muscle function differed between healthy volunteers and Crohn's disease patients relative to leg lean mass. We were unable to complete DEXA scanning on 3 volunteers (2CD) thus body composition and muscle function data are presented for the remaining 33 volunteers (19 CD).

### 5.3.2 Muscle function

Muscle strength was quantified as the peak value obtained during three individual maximal voluntary isometric contractions of the knee extensor muscles. Fatiguability was quantified by calculating the sum work performed during a series of 20 repeated maximum isokinetic knee extensions.

### 5.3.3 Statistical analyses

All data were analysed in GraphPad Prism 7 and are presented as Mean ± SD. Data were checked for normality using a Shapiro-Wilk normality test and analysed by a parametric independent samples *t*-test (healthy volunteers vs Crohn's disease patients). Where data were not normally distributed, a Mann-Whitney test was performed. Statistical significance was set at $P < 0.05$.

With respect to sample size calculations, lower limb isometric strength is reported at 35.8 ± 6.7 N/kgffm in healthy controls vs 28.8 ± 6.4 in quiescent CD patients ($P < 0.001$) [10]. Assuming a power of 80% and $\alpha$=0.05, 17 subjects in each group would be required to show differences in isometric thigh strength between healthy controls and quiescent CD patients.

Lower limb endurance is reported at 26.5 ± 5.8 in healthy controls vs 21.1 ± 5.2 N/kgffm ($P <$ 0.001) in quiescent Crohn's disease patients [10]. Assuming a power of 80% and $\alpha$=0.05,15 subjects in each group would be required to show difference in lower limb fatiguability between healthy controls and quiescent CD patients.

## 5.4 Results

### 5.4.1 Baseline characteristics

The healthy volunteer cohort and Crohn's disease group were well matched for age ($P$ = 0.36) and BMI ($P$=0.64). The magnitude of self-reported fatigue perception the general domain was significantly greater in Crohn's disease relative to healthy volunteers (9 ± 3 vs 14 ± 4, $t_{34}$ = 3.81, $P$ = < 0.001). The 95% CI for the mean of the difference was from 2.26 to 7.43. However, the perception of fatigue in the physical domain did not significantly differ between groups ($P$ = 0.13).Fatigue perception was also assessed using the IBD Fatigue scale in the Crohn's disease patients only. Mean fatigue scores were 9 ± 5 in section one and 33 ± 23 in section two. Crohn's disease patients did not differ in anxiety and depression symptoms (HADS Score, 8 ± 5 vs 10 ± 6, $P$ = 0.35) or cognitive function assessed by the MoCA ( 26 ± 3 vs 26 ± 3, $P$ = 0.80). Mean daily step-count was significantly less in Crohn's disease patients relative to healthy volunteers (8168 ± 3891 vs 5479 ± 2492, $t_{27}$ = 2.27, $P$ = < 0.03) (Table 5-1 & Figure 5-1).

| Demographics | | | |
|---|---|---|---|
| | HV (N=15) | CD (N=21) | *P* - Value |
| Age | 35 ± 12 | 41 ± 14 | 0.31 |
| BMI | 23.7 ± 3.2 | 24.2 ± 3.8 | 0.76 |
| **Clinical characteristics** | | | |
| HBI Score | N/A | 2 ± 2 | N/A |
| Disease duration (years) | N/A | 14 ± 12 | N/A |
| Aminosalicylates (%) | N/A | 19.1 | N/A |
| Immunomodulators (%) | N/A | 23.8 | N/A |
| Previous bowel resection (%) | N/A | 28.6 | N/A |
| **Fatigue measurements** | | | |
| MFI General | 9 ± 3 | 14 ± 4 | 0.0006*** |
| MFI Physical | 8 ± 3 | 11 ± 5 | 0.13 |
| IBDF Scale (S1) | N/A | 9 ±5 | N/A |
| IBDF Scale (S2) | N/A | 33 ± 23 | N/A |
| **Mental health, cognitive function and habitual physical activity measurements** | | | |
| HADS | 8 ± 5 | 10 ± 6 | 0.35 |
| MoCA | 26 ± 3 | 26 ± 3 | 0.80 |
| Step count | 8168 ± 3891 | 5479 ± 2492 | 0.0312* |

***Table 5-1.*** *Demographic data, clinical characteristics including disease activity (Harvey Bradshaw Index) disease duration, current medications and history of bowel resection expressed as a % of the patient cohort. Multiple Fatigue Inventory 20(MFI-20) fatigue scores. IBD Fatigue scale scores. Depression and anxiety symptoms (HADS) (hospital anxiety and*

*depression scale (HADS) scores, Cognitive function scores (MoCA) and mean daily step-count measurements in healthy volunteers and Crohn's disease patients.*

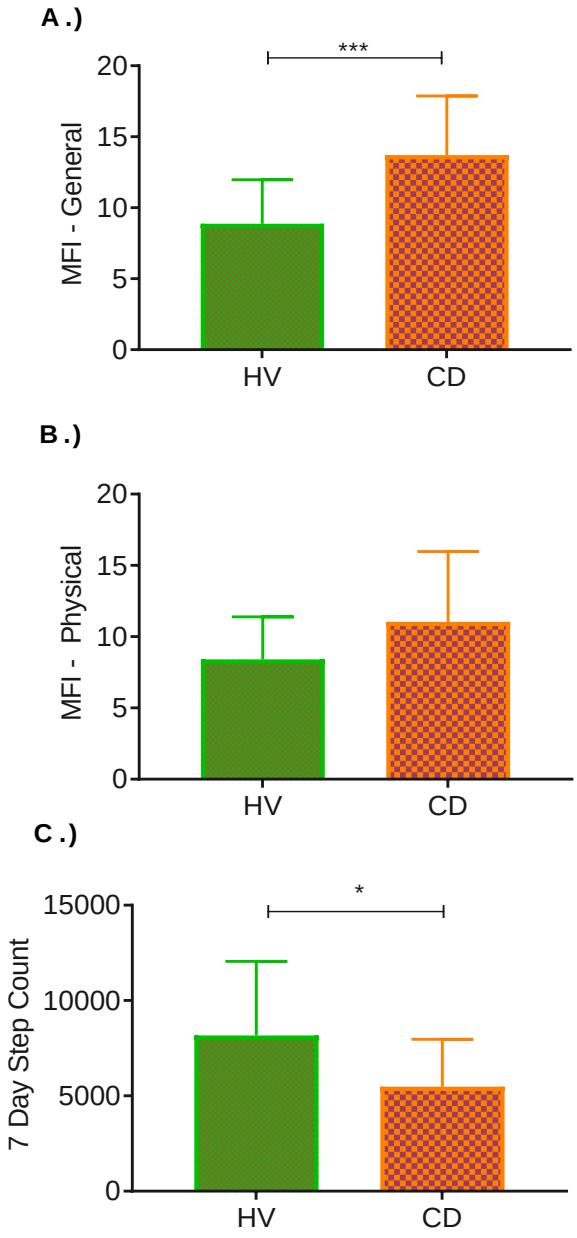


***Figure 5-2.*** *General and physical trait level fatigue reported on the multidimensional fatigue inventory 20 (MFI-20) scale and mean daily 7-step-count in healthy volunteers and quiescent Crohn's disease patients.*

### 5.4.2 Body composition

Total body lean mass was consistent between healthy volunteers and Crohn's disease patients (*P* = 0.73). This was consistent when calculating lean mass index (*P* = 0.78) and appendicular lean mass index (*P* = 0.62). Tissue fat percentage was also comparable between the two groups (*P* = 0.63) as was trunk fat mass (*P*=0.93) and BMD (*P* =0.73) (Table 5-2 & Figure 5-2).

| | HV (N=14) | CD (N=19) | *P* value |
|---|---|---|---|
| Total lean mass (kg) | 48.29 ± 13.31 | 45.64 ± 8.41 | 0.73 |
| LMI | 15.97 ± 2.74 | 15.55 ± 1.61 | 0.78 |
| ALMI | 7.14 ± 1.38 | 6.94 ± 0.95 | 0.62 |
| BMD ($g/cm^2$) | 1.19 ± 0.12 | 1.21 ± 0.10 | 0.73 |
| Tissue Fat (%) | 30.10 ± 8.30 | 31.37 ± 7.50 | 0.63 |
| Trunk fat mass (kg) | 10.25 ± 3.82 | 10.11 ± 4.51 | 0.93 |

***Table 5-2****. Absolute total body lean mass, lean mass index (total body lean mass /$ht^2$), appendicular lean mass index (appendicular lean mass/$ht^2$) relative total body fat mass % in age and BMI matched healthy volunteers and Crohn's disease patients. One HV data set lost (N=14) due to scan failure.*

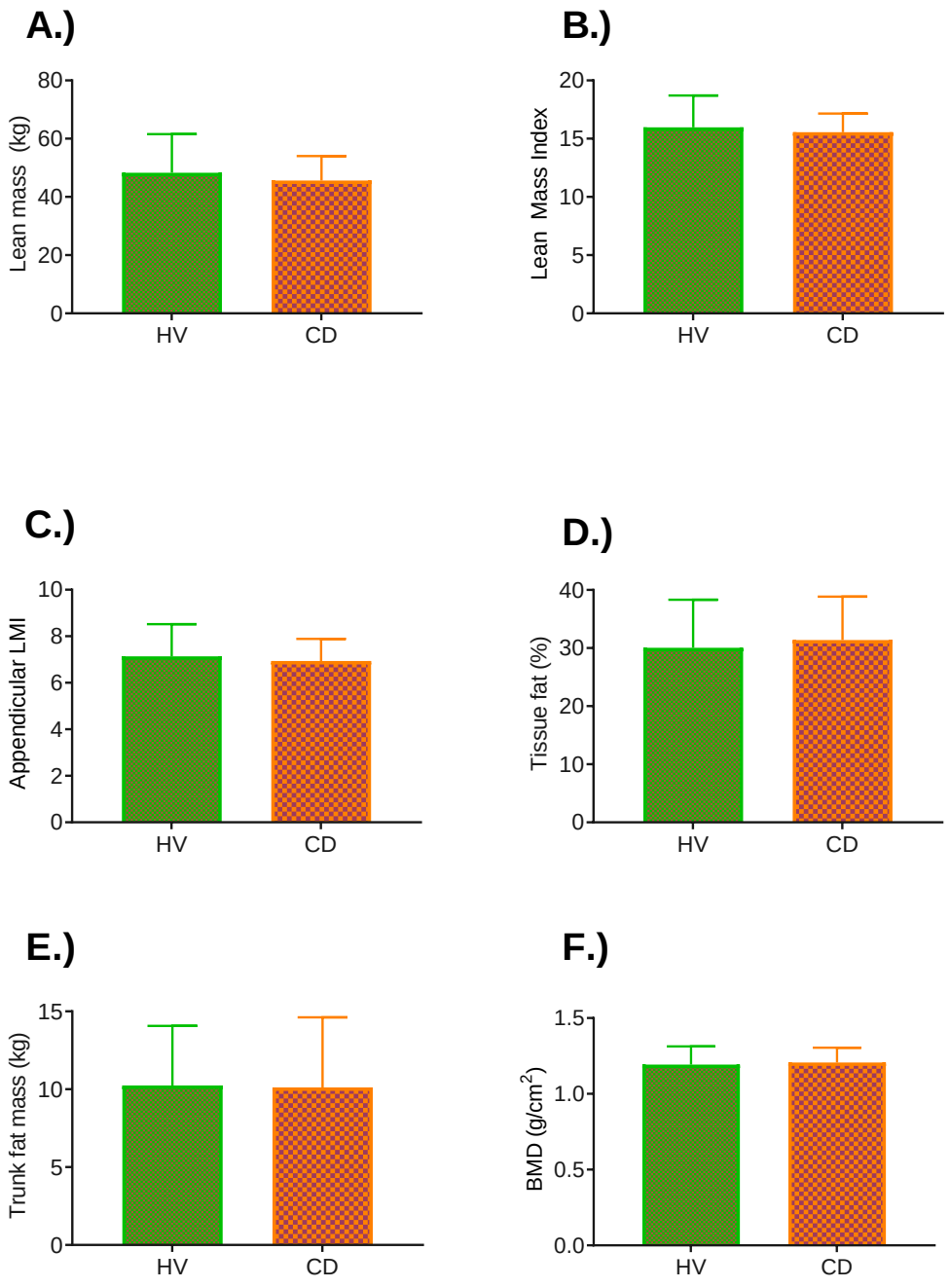


***Figure 5-3**. Total lean mass (kg), Lean mass index (total body lean mass /ht$^2$), appendicular lean mass index (appendicular lean mass/ht$^2$), Total tissue fat %, Trunk fat mass and bone mineral density in healthy volunteers and Crohn's disease patients.*

### 5.4.3 Muscle function

Knee extensor isometric strength was no different between healthy volunteers and Crohn's disease patients when normalised to leg lean mass (12.78 ± 2.87 vs 12.61 ± 1.93, $t_{32} = 0.21$, $P = 0.84$). The 95% CI for the mean of the difference was from -1.85 to 1.51.

Total isokinetic work performed during 20 repeated maximal isokinetic knee extensions was no different between healthy volunteers and Crohn's disease patients when normalised to leg lean mass (138.2 ± 22.31 vs 133.7 ± 19.08, $t_{32} = 0.63$, $P = 0.53$) The 95% CI for the mean of the difference was from -19 to 10. Results are displayed in table 5-3 and figure 5-4.

| | HV (N=14) | CD (N=19) | *P* value |
|---|---|---|---|
| Strength (Nm/kg leg lean tissue mass) | 12.78 ± 2.87 | 12.61 ± 1.93 | 0.84 |
| Work (Nm/ kg leg lean tissue mass) | 138.2 ± 22.31 | 133.7 ± 19.08 | 0.53 |

***Table 5-3.*** *Maximum isometric thigh strength (NM) and total work performed (NM) during 20 maximal repeated isokinetic contractions normalised to leg lean tissue mass.*

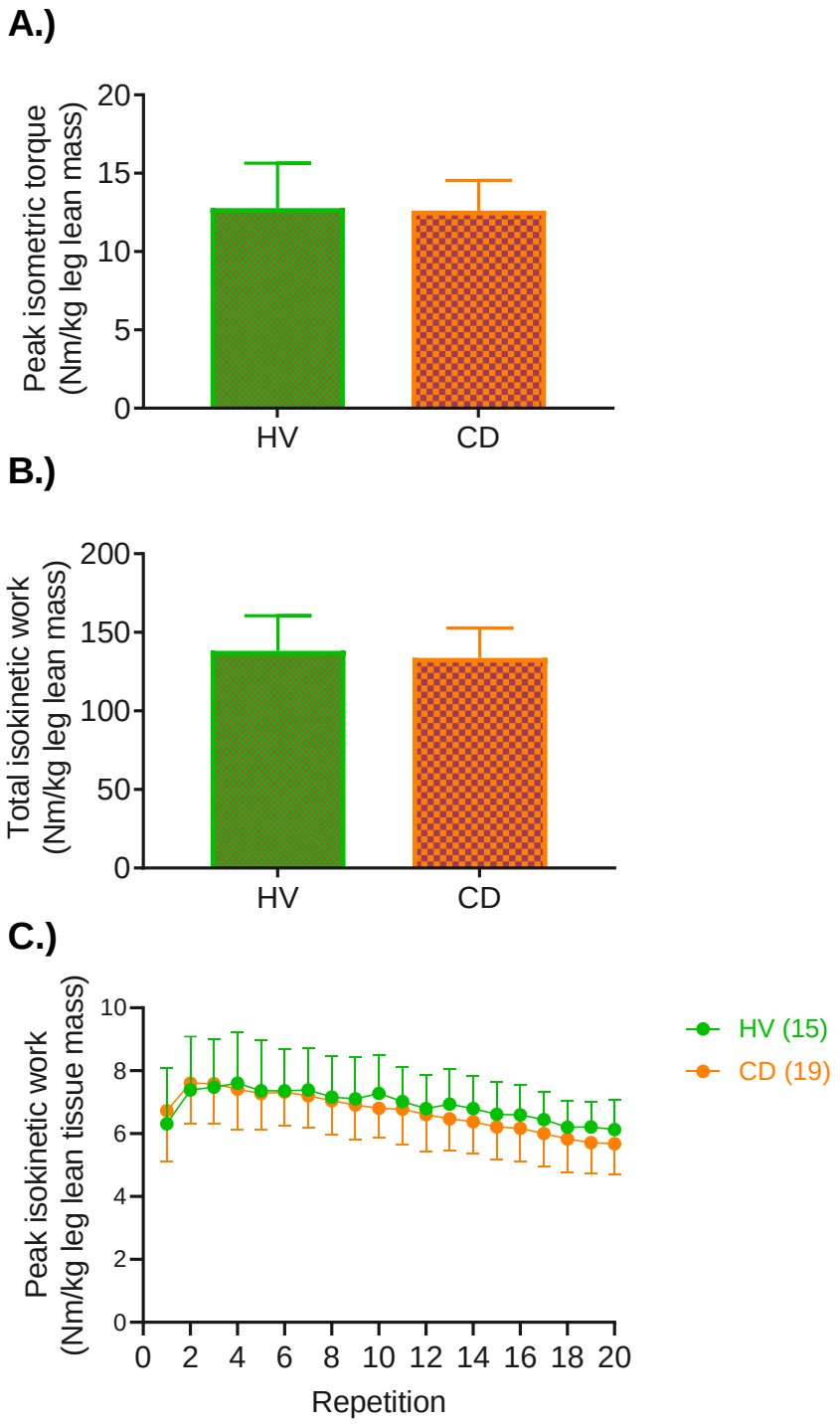


***Figure 5-4.*** *Thigh maximum isometric strength normalised to leg lean mass (Nm/kg) (A.), lean Total isokinetic work performed during 20 repeated maximal contractions normalised to leg lean mass (Nm/kg) (B.),Peak isokinetic work per contraction normalised to leg lean mass (Nm/kg). Values represented as mean ± SD. Error bars plotted in single, opposite directions to aid in data visualization.*

## 5.5 Discussion

The main purpose of this work was to establish whether quiescent Crohn's disease patients self-report an increased perception of fatigue, present with altered body composition, peripheral muscle weakness and premature fatiguability during maximal voluntary contractions, relative to age and BMI matched healthy volunteers. A comparison of habitual physical activity levels measured by pedometer tracked step-count was also carried out.

### 5.5.1 Self-reported fatigue perception

In line with literature recommendations [21, 22] and previous experiments in IBD [23, 24], we chose to report the mean fatigue perception scores from the general and physical domain of the MFI-20. Consistent with the existing body of data on self-reported fatigue perception in IBD, the quiescent Crohn's disease group reported significantly greater fatigue perception relative to healthy controls, as assessed by the general domain of the MFI-20 scale [25]. However, fatigue perception scores on the physical domain of the MFI-20 scale were comparable between Crohn's disease patients and healthy controls. In previous data in IBD utilizing the MFI-20 scale to assess fatigue perception, mean fatigue scores in the general and physical domain were reported at 14.2 ± 4.3 and 13.6 ± 4.3 in 124 female Crohn's disease patients with mixed disease activity. Similarly, male patient scores were 12.8 ± 4.3 and 12.4 ± 4.4 [26]. In another cohort of 36 quiescent Crohn's disease patients, General fatigue was reported at 10.5 ± 0.71 and Physical 9.03 ± 0.64 [25] whilst more recent data demonstrate general fatigue scores were 11.8 ± 4.4 and physical 10.3 ± 3.9 in 39 Crohn's disease patients with no self-reported disease activity [27].

Previous IBD experiments have defined fatigue by calculating a threshold based on the 95$^{th}$ percentile of the general fatigue scores on the MFI-20 in the general population [24-26] or an internal healthy control group [25]. This equates to a fatigue score of ≥13, whilst others have

used the 75$^{th}$ percentile of general population [28] scores [23] equating to a fatigue score of ≥11. Currently these approaches are the only way to ascertain clinically relevant fatigue burden, which remains a general challenge in the field [29, 30]. Based on these variable previously defined cut offs, clinically significant fatigue prevalence would have ranged between 62-67% in our quiescent Crohn's disease cohort based on an MFI general score of ≥11 or ≥13. Which is greater than previously reported in a quiescent [25] and mixed disease cohort of Crohn's disease patients [26].

Regarding IBF Fatigue scale scores, given the relatively sparse use of this fatigue instrument across the available body of literature, it is difficult to reconcile the few available data to draw firm conclusions pertaining to self-reported fatigue burden using this scale. The IBDF scale scores in this quiescent cohort of 9 ± 3 (Section 1) and 33 ± 23 (Section 2) are relatively comparable to baseline IBDF scores previously reported at 11.93 ± 3.24 and 9.93 ± 4.13 on section one in the intervention and control groups respectively. Although our patient S2 scores were lower than the corresponding S2 scores of 55.83 ± 26.74 and 49 ± 28.66 reported in two subgroups of IBD patients prior to a cognitive behavioural therapy intervention aimed at targeting fatigue perception [31].

In another cohort, mean section one scores were reported at 6 ± 4 in the intervention group and 9 ± 4 in the control group prior to a resistance training intervention [32]. Again, these are comparable to the data presented in this chapter. The application of the IBD Fatigue scale in the general population found S1 scores of 7 ± 3.9 and 15.3 ± 17.4 in a cohort of 1761 participants [33]. Clearly, there appears to be little difference in S1 scores between various IBD cohorts and the general population. Conversely, the S2 scores reported in our quiescent patients do appear to be less than available IBD patient data [31]. This is consistent with the variable fatigue scores reported on the MFI-20 in our data and is implicit of lower fatigue burden in our quiescent cohort. This could be considered consistent with these patients volunteering for this

strenuous exercise study and raises the question of whether quiescent patients with the greatest perceived fatigue burden were adequately captured within our sample.

### 5.5.2 Body composition

In agreement with a large proportion of available data in quiescent IBD, we report a maintenance of body composition relative to healthy controls in our cohort of Crohn's disease patients. All indices of lean mass whether assessed as absolute total body values, or total body / appendicular values normalised to height (i.e. Lean mass index or appendicular lean mass index [20]), were consistent with that observed in healthy age and BMI matched controls. Both tissue fat % and BMD were also no different between the healthy volunteers and Crohn's disease patients.

The lack of difference in body composition in our cohort of Crohn's disease patients likely relates to the deep remission status of the patients and associated improvements in dietary intake, physical conditioning and general health status, relative to an active disease state. Treatment related factors may have also influenced this. For example, infliximab therapy has been shown to increase muscle cross sectional area and strength in IBD [34]. Furthermore, patients with current or recent corticosteroid prescriptions (within the last 12 weeks prior to recruitment) had been excluded from trial participation, owing to the negative effects of glucocorticoids on skeletal muscle. Glucocorticoids are also associated with increased adiposity [35], thus the sole inclusion of steroid free Crohn's disease patients may explain the maintenance of body composition in this cohort. In further support of this, we also reported a maintenance of bone mineral density relative to the healthy control group. This appears to be a rare finding in Crohn's disease, where bone density is generally shown to be reduced relative to healthy controls patients. A systematic review found that bone density was reduced in 87% of Crohn's disease patients and maintained relative to healthy controls in only two individual

experiments across the available literature [19]. Glucocorticoid treatment is a known risk factor for reduced bone density [36] due to the negative effects on bone metabolism [37]. However, both lean mass and fat mass exert mechanical stress upon the skeleton and strongly influence BMD, where lean mass is a greater contributor than fat mass under physiological conditions [38]. Thus, the comparable bone mineral density in our cohort is consistent with the maintenance of body composition observed relative to healthy controls. Interestingly, reduced bone density can also be targeted with infliximab therapy, where regular administrations at 6-8 week intervals increased bone mineral density in Crohn's disease patients over a one year measurement period [39].

### 5.5.3 Muscle strength

During sustained maximum voluntary contractions it is known from the application of electrophysiological techniques, that the majority of force loss (i.e. exercise fatigue) in healthy young, well-motivated subjects, is due to mechanisms directly at the skeletal muscle level [40]. Whereas, during submaximal dynamic exercise [41], centrally mediated brain fatigue arising from reduced motor cortical output accounts for 50-66% of exercise fatigue [42]. This is consistent with the correlation between strength and muscle mass [15] and between DEXA estimated lean body mass and thigh strength in both healthy control's and quiescent IBD patients [16]. Therefore, our observation of sustained maximum isometric knee extensor strength between Crohn's disease patients and healthy volunteers is congruent with the maintenance of lean body mass, of which lower limb lean mass is a significant contributor. Interestingly, a maintenance of maximal isometric knee extensor strength has previously been reported in IBD patients with self-reported fatigue symptoms [13] despite these patients presenting with less thigh cross sectional area (-14%, $P = 0.055$) and aberrant hypertrophy signalling in skeletal muscle [43] relative to a healthy control group.

### 5.5.4 Muscle fatiguability

Comparison of the total isokinetic work performed during a series of 20 maximal isokinetic thigh contractions between Crohn's disease patients and healthy volunteers revealed no between group differences. Aforementioned data used to infer knee extensor fatiguability in IBD has been derived from assessments more closely related to muscle strength capabilities than fatiguability (i.e. the ability of skeletal muscle to repeatedly produce maximal instantaneous force as appose to sustaining a given work output over time) thus making comparisons with our data inappropriate.

### 5.5.5 Habitual physical activity

Despite the maintenance of body composition alongside peripheral muscle strength and fatiguability, the habitual physical activity of our Crohn's disease patients was significantly less than in healthy control volunteers. These findings are in agreement with available data on physical activity levels in IBD [11, 43-45] . Reduced physical activity is known to negatively affect skeletal muscle [46]. Consistently, Crohn's disease patients with a low phosphorylated: total Akt ratio, performed less physical activity relative to patients with a high ratio ($P = 0.009$). The combined cohort also had less thigh muscle mass than healthy controls ($P = 0.055$) [43]. Whilst the habitual physical activity undertaken by our Crohn's disease cohort was significantly less than in healthy controls, this reduction does not appear to be of a great enough magnitude to adversely affect muscle mass and strength. Given that whole-body lean mass, muscle strength and fatiguability were no different between groups. The mean step count for patients (~5400/day, Figure 1, C & Table 1) is comparable to baseline step counts reported in patients with osteoarthritis of the knee (4652 ± 2622) [47] and joint arthroplasty (5482 ± 150) [48]. This step count is consistent with the "low active lifestyle" outlined by the step-defined

sedentary lifestyle index (5000-7499 step/day) [49]. It is also significantly lower than the minimum step count recommended by the American College of Sports Medicine, for maintaining general health status (≥7000 steps/day) [50]. A reduction in habitual physical activity and maintenance of body composition has been reported previously in patients who display premature exercise fatigue during dynamic tasks [11]. It is likely that the deconditioning associated with physical inactivity (i.e. a loss of muscle mitochondrial content) is a strong contributor to these functional losses seen on dynamic task performance [10, 11] and incremental exercise testing [2, 51, 52].

### 5.6 Conclusion

These collective findings evidence a maintenance of body composition, bone density and peripheral muscle strength in quiescent Crohn's disease patients with varying fatigue perception scores, relative to healthy volunteers. This was observed despite a reduction in habitual physical activity. Collectively, the well-controlled disease in our Crohn's disease cohort, together with the exclusion criteria of glucocorticoid prescriptions and inclusion of patients receiving biological therapies, may explain these findings.

These data indicate that perceived fatigue burden in quiescent IBD is variable depending on the specific domain of assessment and appears unrelated to atrophy, peripheral muscle weakness and premature muscle fatiguability. This suggests that laboratory-based assessments of peripheral muscle strength, which provides information predominantly on neural drive and instantaneous muscle mass recruitment or the ability to sustain maximum force output during fatigue tests, are likely not suited to objective fatigue assessment in IBD. Particularly in the high proportion of patients with maintained body composition relative to healthy controls [19]. Generally, these assessments are unrelated to everyday task performance that fatigued IBD patients report difficulties with [23]. These tasks are also mechanistically divergent from

repeated submaximal contractions during traditional dynamic exercise modes, where performance is known to be reduced in IBD. This includes repeated sit up and stand assessments [10, 11] gait speed [11], and aerobic exercise performance [2, 51, 52]. These findings together with the data in this chapter point to deconditioning (i.e. reduced $VO_2$ peak) as a potential aetiological factor in the increased fatigue perception in IBD, as appose to muscle weakness and premature muscle fatigue. This will be investigated in the following chapter.

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# Chapter 6. Whole-body deconditioning in quiescent Crohn's disease patients.

**Abstract**

Following the assessment of self-reported fatigue perception, body composition and peripheral muscle function in quiescent Crohn's disease patients, chapter 6 aimed to assess whether these patients demonstrated central deconditioning relative to age and BMI matched healthy volunteers.

Cardiorespiratory fitness was assessed by supine incremental exercise to volitional exhaustion. Subsequently, cardiac output, cerebral blood flow and oxygen metabolism was assessed during sustained low-intensity stepping exercise at 50% supine $VO_2$ peak.

Supine $VO_2$ peak and all other CPET parameters achieved at peak workload, were no different between the Crohn's disease patients and healthy volunteers.

During supine stepping at 50% $VO_2$ peak, cardiac output was no different between Crohn's disease and healthy volunteers. Cerebral blood flow increased on exercise in the healthy volunteers ($P < 0.05$) but not in the Crohn's disease patients. The cerebral oxygen metabolism response across the exercise task was no different between groups.

The collective findings from this chapter provides no evidence of central cardiorespiratory deconditioning in quiescent Crohn's disease patients with a range of self-reported fatigue perception, relative to healthy volunteers.

## 6.1 Introduction

Chapter 5 provided evidence that body composition, peripheral muscle strength and fatiguability do not differ between a cohort of quiescent Crohn's disease patients with a range of fatigue perception scores and healthy volunteers. Although habitual physical activity was less in the Crohn's disease patients compared to the healthy volunteers. Next, we sought to assess whether these Crohn's disease patients demonstrated whole-body deconditioning assessed by $VO_2$ peak during supine cardiopulmonary exercise testing.

A review of available data on whole-body deconditioning in IBD can be found in Chapter 2. This revealed clear evidence that cardiorespiratory fitness is reduced in IBD compared to healthy controls [1, 2], and that this reduction appears to be of a greater magnitude in patients with an increased perception of fatigue [3].

Currently, there is a complete absence of mechanistic data pertaining to the whole-body deconditioning present in IBD. In Crohn's disease, it is unknown whether the exercise fatigue during both laboratory based incremental exercise testing [1-3] and dynamic lower limb performance [4, 5] occurs due to alterations in central or peripheral physiology [6]. During sustained exercise performance, there is a tight interlink between the cardiorespiratory system, central nervous system and skeletal muscle [7]. Alterations in any of these systems such as reduced neural drive to skeletal muscle, or a reliance on intramyocellular anaerobic ATP synthesis can thus potentiate exercise fatigue.

This chapter aims to assess the potential contributions of central deconditioning to IBD fatigue aetiology by comparing proton ($^1$H) MRI, PC-MRI and TRUST acquisitions in a quiescent CD cohort to a HV cohort across a low-intensity aerobic exercise task (see Chapter 4 for a detailed description of MR methods and experimental protocols).

## 6.2 Aims

In this chapter we have undertaken cardiopulmonary exercise testing to quantify whole-body deconditioning in a cohort of quiescent CD patients with a range of fatigue perception scores (add data). This was followed by ($^{1}$H) MRI phenotyping of the quiescent CD group compared to HVs during within-bore aerobic exercise challenge. These data aim to investigate the central cardiovascular and neurovascular contributions to the premature fatiguability previously reported in IBD cohorts, which is thus far absent within the available literature.

To determine the role of central deconditioning to IBD fatigue aetiology, cardiac output, cerebral blood flow and brain fractional oxygen extraction were assessed at rest, during sustained low-intensity supine stepping exercise and on recovery from exercise. Comparison were made between quiescent CD patients with a range of fatigue perception scores relative to an age and BMI matched healthy control cohort.

## 6.3 Methods

### 6.3.1 Experimental Protocol

The data presented within this chapter were obtained over two study visits as outlined in detail in Chapter 4. Briefly, following cardiopulmonary exercise testing at the David Greenfield Human Physiology Unit (DGHPU), HV and CD participants completed a second MR study visit at the Sir Peter Mansfield Imaging Centre (SPMIC). The first component of the MR study day comprised MRI assessment of central responses (cardiac, brain vascular and metabolic) to low-intensity supine stepping exercise performed at 50% $VO_2$ peak, as established during Visit one. This was performed on the wide-bore 3T Ingenia scanner. An outline of the theory behind the MRI sequences can be found in Chapter 4.

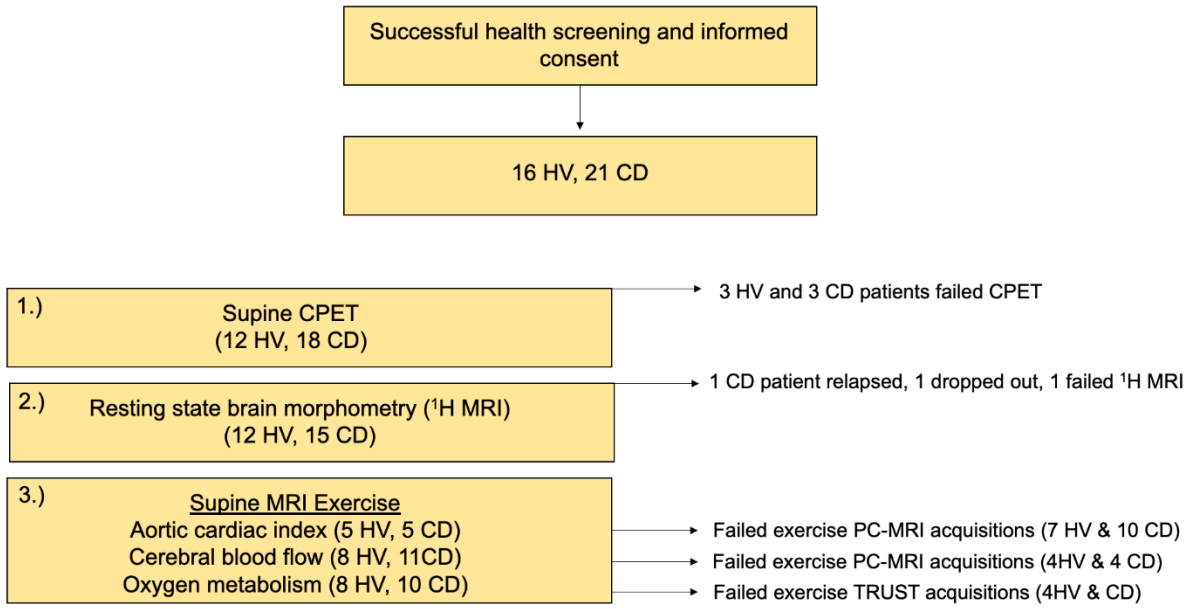


***Figure 6-1:*** *Sample sizes relating to successful completion of experimental assessments followed by stratification of sample sizes for individual MRI and $^{31}P$ MRS acquisitions. Details of metabolic parameters calculated from non-localised*

### 6.3.2 Central MRI sequences

Blood flow (velocity and flux) in L/R internal carotid arteries (ICA) and basilar artery (BA) was measured using a vectorcardiogram (VCG) gated, 2D PC-MRA (TR/TE = 15/6.5 ms, FA = 25°, FOV = 280 x 77 mm2, 0.75 x 0.75 x 6 mm3 reconstructed, SENSE 4, $v_{ENC}$ = 0 and 100 cm/s, scan duration = 1 min 25s, NSA = 2). Venous oxygenation, Yv, in the superior sagittal sinus (SSS) was quantified using a T2 Relaxation Under Spin Tagging (TRUST) sequence [8], as described in Chapter 4. The TRUST imaging slice was positioned parallel to the anterior commissure–posterior commissure line and perpendicular to direction of flow in the SSS vein. Scan parameters comprised an in-plane field of view (FOV) of 230 mm x 230 mm, spatial resolution 3.44 x 3.44 mm2, slice thickness 5 mm, TI = 1022 ms, and TR = 3000 ms per

label/control pair. Four eTEs were collected at 1, 40, 80, 160 ms corresponding to 0, 4, 8 and 16 refocussing pulses in the T2 preparation with an interval cpmg = 10 ms (Carr-Purcell-Meiboom-Gill).

A high-resolution whole-brain T1-weighted MPRAGE sequence was acquired to assess grey matter volume. Scan parameters were TR = 8.39ms, TE = 3.86ms, flip angle = 8º, 160 contiguous slices, slice thickness = 1mm, FOV 256 x 256 mm2, spatial resolution 1mm isotropic, NSA = 1.

To determine cardiac output, flow quantification of the ascending aorta during free breathing was performed. Blood flow was measured through a transversal plane at the height of the pulmonary bifurcation perpendicular to the ascending aorta with VCG-triggered phase-encoded velocity-mapping sequences. Imaging parameters were FOV = 280 x 77mm$^2$, spatial resolution = 0.75 x 0.75mm2, slice thickness 6mm, SENSE factor 3, TE/TR = 6.5/15ms, FA = 25°, VENC 100cm/s, NSA = 2. The sequence was cardiac triggered using the PPU with the data acquired at 30 time points across the cardiac cycle.

### 6.3.3 Data Analysis

PCA data was analysed using Q-Flow (Philips) to determine vessel area, velocity and flux in the left and right ICA and VA, flux measures were summed to estimate total brain blood flow.

PC-MRI data were analysed using the View forum software (Philips Medical Systems, Netherlands). The internal carotid arteries and basilar artery were manually identified, and an ROI was drawn around the walls of each vessel based on the magnitude image. This ROI was propagated through all time points across all phases in the cardiac cycle, with the ROI manually inspected and edited where necessary. Blood velocity (mm/s), and vessel cross sectional area (mm$^2$) were directly measured, and the product of velocity and area within the ROI

($velocity \times area$) computed to calculate flux in each vessel, these were then combined across vessels to compute global cerebral blood flow. To account for brain atrophy, cerebral blood flow was corrected for grey matter volume, gmCBF. Grey matter volume was calculated using tissue segmentation in FSL (FMRIB, UK) from the MPRAGE image. CBF was then normalised by each individual's GM volume.

TRUST data were processed using a dedicated MATLAB script following the methods described in Lu et al [8]. Venous blood signal in the superior sagittal sinus (SSS) was computed from the pairwise subtraction of label and control images to obtain difference images for each effective echo time (eTE). In the resultant difference images, an ROI of four voxels with the highest difference signal created a mask for spatial averaging of the signal. Blood $T_2$ relaxation was then calculated within the SSS ROI by fitting the data to a mono-exponential signal decay. The blood $T_2$ relaxation time in the SSS was then converted into venous oxygenation, *Yv*, using a calibration plot. For a more detailed description see Chapter 4.

To determine cardiac output, the ascending aorta was outlined in the anatomic images for each time point with flow calculation performed in the corresponding velocity-encoded phase images as for the brain PC-MRI measures. The average flow velocity (cm/s) was multiplied by the area of the vessel ($cm^2$) to obtain flow (ml/s) at each time point. Flow throughout the cardiac cycle was integrated over one cardiac cycle to obtain the stroke volume (SV). Cardiac output (CO) was determined as SV multiplied by heart rate (SV x HR), and Cardiac index (CI) calculated from the CO divided by body surface area (BSA).

### 6.3.4 Central vascular and metabolic responses to exercise.

Arterial oxygenation, $Y_a$ was calculated as described by Peng et al [9]

$$Y_a = 99.77 - 0.036 \times age - 1.235 \times sex + 0.021 \times age \times sex \quad (12)$$

where *age* is in years and *sex* is defined as 0 for females and 1 for males [10].

Oxygen extraction fraction was calculated by

$$OEF = Y_a - Y_v \quad (13)$$

gCMRO2 was calculated as described by Liu et al [11]

$$gCMRO_2 = gCBF \times (Y_a - Y_v) \times C_h \quad (14)$$

where *gCMRO2* and *gCBF* are global cerebral metabolic rate of oxygen and global cerebral blood flow (gCBF) and $C_h$ is the capacity of blood to transport oxygen. $C_h$ values were calculated for each subject by converting their individual haemoglobin concentrations obtained during routine blood testing (g/dL) to (mmol/L). When individual haemoglobin values were unavailable, a literature value was assumed (8.34 mmol/L or ~13.4g/dL Hemoglobin). $C_h$ was then adjusted for age assuming a decline rate of 0.0079 mmol/L per year [12].

gmCMRO$_2$ was then corrected for brain atrophy to account for any disease or age related loss of grey and white matter between groups [9].

$$\text{gmCMRO}_2 = \frac{tCMRO_2}{V_{gm} + rV_{wm}} \times \frac{1}{\rho} \quad (15)$$

where *gmCMRO$_2$* is the *CMRO$_2$* normalised to grey matter volume, $V_{gm}$ and $V_{wm}$ are the grey and white matter volumes, r is the ratio between white and grey matter volume and ρ is a constant relating to the mass density of tissue, which is assumed to be 1.06 g/ml [13]

## 6.4 Statistical Analysis

Statistical analyses of the data were performed using IBM SPSS Statistics Version 26. Data were checked for normality by visual inspection of normality plots. Between group comparisons of single dependent variables were carried out by independent samples *t*-test. Between group comparisons across the within-bore exercise tasks were carried out by a Two-way mixed design ANOVA with a Bonferroni correction, to assess the main effect of group (HV vs CD), time (baseline, exercise, recovery) and the combined effects of both factors. Sphericity was assessed by Mauchly's test of sphericity. If the Greenhouse-Geisser epsilon was ≥0.75, the Huynh-Feldt corrected value was used to correct the degrees of freedom, else the Greenouse-Geisser correction was used.

With respect to sample size estimations, the $VO_2$ max of a healthy volunteer is 3.42 ± 0.71 L/min [14]. The $VO_2$ peak of Crohn's disease patients is reported at 2.43 ± 0.75 L/min. Assuming a power of 80% and α=0.05, 9 subjects in each group are needed to show a significant difference between the healthy volunteers and Crohn's disease patients.

It is not possible to power this exact MRI study as pilot data in these IBD cohorts has not been collected. However, we have shown that brain perfusion in the young increases by 18 ± 6% during supine cycling at 30% $VO_2$max . Whilst in the elderly, it increases by only 3 ± 4% [15, 16]. If we assume a power of 100% and α=0.05, 10 subjects in each group are needed to show a significant difference between healthy volunteers and a potentially fatigued cohort [15, 16]. Further, for neuroimaging studies it is generally considered that a minimum of 12 subjects is needed in each cohort [17].

## 6.5 Results

To aid in clarity of data interpretation, group sample sizes for the experimental parameters covered within this results section are detailed in figure 6-1. Given the difficulty of obtaining

certain $^{1}$H MRI measurements during within-bore exercise (e.g. motion artefacts preventing data analysis) the final sample sizes reflecting successful acquisition of the relevant MRI sequences are also detailed.

### 6.5.1 Baseline characteristics

A total of 12 HV and 19 CD patients completed both the functional laboratory-based exercise assessments and the subsequent MRI exercise challenge. The demographic data for these patients is reported in table 6-1. These cohorts remained well matched for age ($P$ = 0.85) and BMI ($P$ > 0.99). Self-reported fatigue status remained significantly greater in the general domain of the MFI-20 scale in Crohn's disease patients relative to healthy volunteers (14 ± 4 vs 8 ± 3, $t_{28}$ = 1.78, $P$ = 0.0009). The 95% CI of the mean of the difference was from -0.43 to 6.13. However, , fatigue scores were comparable on the physical domain of the MFI-20 ($P$ = 0.086).Similarly, the IBDF scale fatigue scores were 9 ± 6 on scale 1 and 34 ± 24 on scale two. Habitual daily step count also remained significantly greater in the healthy volunteers relative to the Crohn's disease patients ( 8168 ± 3891 vs 5482 ± 2648, $t_{25}$ = 2.13, $P$ = 0.0431. The 95% CI for the mean of the difference was from -5282 to -90.82.

| Demographics | | | |
|---|---|---|---|
| | HV (N=12) | CD (N=19) | *P* - Value |
| Age | 38 ± 13 | 39 ± 14 | 0.85 |
| BMI | 23.9 ± 3.2 | 24.2 ± 4 | > 0.99 |
| **Clinical characteristics** | | | |
| HBI Score | N/A | 2 ± 2 | N/A |
| Disease duration (years) | N/A | 12 ± 12 | N/A |
| Aminosalicylates (%) | N/A | 21.05 | N/A |
| Immunomodulators (%) | N/A | 26.32 | N/A |
| Previous bowel resection (%) | N/A | 26.32 | N/A |
| **Fatigue measurements** | | | |
| MFI General | 8 ± 3 | 14 ± 4 | 0.0009 |
| MFI Physical | 8 ± 3 | 11 ± 5 | 0.086 |
| IBDF Scale (S1) | N/A | 9 ± 6 | N/A |
| IBDF Scale (S2) | N/A | 34 ± 24 | N/A |
| **Habitual physical activity measurements** | | | |
| Step count | 8168 ± 3891 | 5482 ± 2648 | 0.0431 |

***Table 6-1 .*** *Demographic data, clinical characteristics including disease activity (Harvey Bradshaw Index) disease duration, current medications and history of bowel resection expressed as a % of the patient cohort. Multiple Fatigue Inventory 20(MFI-20), IBD Fatigue scale section one and two scores. Depression and anxiety symptoms (HADS), Cognitive function scores (MoCA) and daily step-count measurements in healthy volunteers and Crohn's disease patients.*

### 6.5.2 Whole-body deconditioning - Cardiopulmonary exercise testing (CPET)

Table 6-2 shows the CPET parameters for the HV and CD group. Details of the incremental exercise testing protocol are described in chapter 4. It can be seen that there was no significant difference in any peak CPET parameters between the HV and CD groups.

| CPET Parameters | HV (12) | CD (18) | *P* Value |
|---|---|---|---|
| **VE (L/min)** | 67.3 ± 16.8 | 64.0 ± 12.8 | 0.98 |
| **$VCO_2$ (L/min)** | 1.94 ± 0.46 | 1.79 ± 0.30 | 0.55 |
| **$VO_2$ (mL/kg/min)** | 25.9 ± 5.1 | 25.0 ± 5.0 | 0.64 |
| **Heart rate (bpm)** | 150 ± 20 | 158 ± 20 | 0.32 |
| **Peak Power (Watts)** | 171 ± 38 | 177 ± 44 | 0.68 |
| **RPE** | 18 ± 1 | 18 ± 2 | 0.87 |
| **Blood lactate (mmol/L)** | 5.0 ± 2.0 | 5.2 ± 1.6 | 0.78 |
| **$R^2$ value** | 0.98 ± 0.01 | 0.97 ± 0.01 | 0.28 |

***Table 6-2.*** *Peak values for minute ventilation, expired $CO_2$, oxygen uptake, heart rate, power, rate of perceived exertion (RPE), arterialised venous blood lactate during supine incremental exercise testing. $R^2$ value from linear regression used to interpolate workload at 50% $VO_2$ peak for within-bore exercise.*

### 6.5.3 Central physiology

| Brain morphometry | HV (12) | CD (15) | *P* Value |
|---|---|---|---|
| **Grey matter volume ($cm^3$)** | 632.6 ± 93.61 | 647.7 ± 70.19 | 0.64 |
| **White matter volume ($cm^3$)** | 519.2 ± 61.04 | 541.8 ± 41.03 | 0.26 |
| **Cerebrospinal fluid (mL)** | 283.8 ± 50.67 | 236.5 ± 24.24 | 0.004 |

***Table 6-3.*** *Brain morphometry data in healthy volunteers and Crohn's disease patients.*

Global brain structure in HV versus CD

Results of brain structural measures from the MPRAGE scan are shown in Figure 6-2. The mean grey matter volume for the CD group was not significantly different that of the HV group (*P* =0.64). The mean white matter volume for the CD group was not significantly different to the HV group (*P*=0.26). In contrast, the mean CSF volume for the CD group (236.5 ± 24.2 ml) was significantly lower ($t_{25}$ = 3.2, *P* = 0.004) than the mean of the HV group (283.80 ± 50.67 ml). The 95% CI for the mean of the difference was from 16.9 to 77.83 ml. (Table 6-3 and Fig. 6-2C)

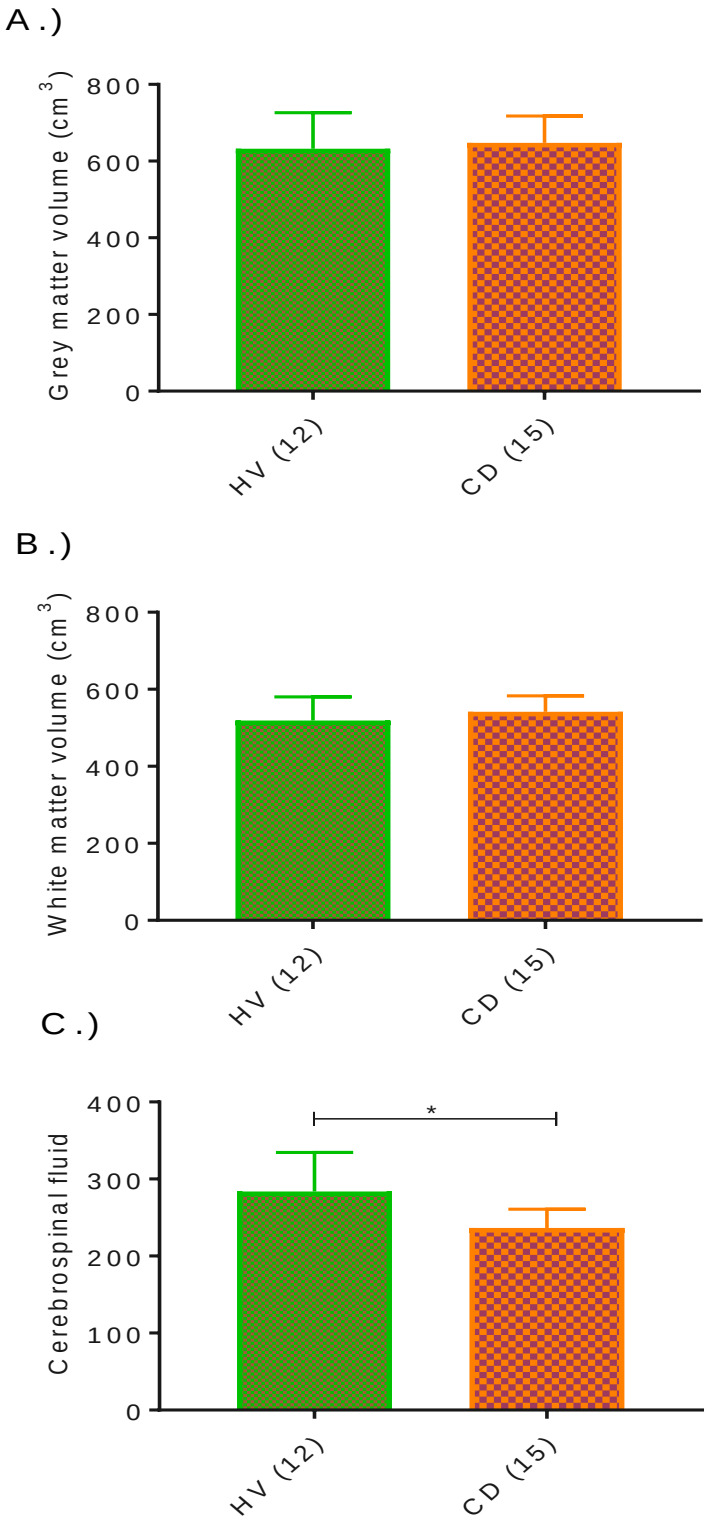


***Figure 6-2.*** *Grey matter, white matter and CSF volumes in the HV and CD groups. A significant reduction in CSF is seen on the CD group compared to HV group.*

### 6.5.4 Aortic Cardiac index

There was no main effect for group ($F_{1, 8} = 0.967$, $P = 0.354$). Cardiac index was consistent between HV and CD across all three time points. There was a main effect for time $F_{2, 16} = 35.041$, $P < 0.001$). Cardiac index significantly increased during supine exercise relative to baseline ( $P < 0.001$) and significantly decreased during exercise recovery, relative to during exercise ( $P < 0.001$). Baseline and recovery cardiac index were consistent ($P = 1.0$). There was no interaction effect ($F_{2,16} = 0.313$, $P = 0.735$). Both groups showed comparable increases in cardiac index during supine exercise and a return to baseline values during recovery from exercise.

% change cardiac index: There was no main effect for group ($F_{1, 7} = 0.279$, $P = 0.613$). The % change in cardiac output on exercise was comparable in HV and CD. There was a main effect for time ($F_{1, 7} = 118.874$, $P <0.001$). The % change in cardiac index was significantly greater on exercise than recovery ($P < 0.01$). There was no significant interaction ($F_{1, 7} = 0.218$, $P = 0.655$) the % increase in cardiac index on exercise was similar between HV and CD as were the % change in recovery values relative to baseline measurements.

% change HR: There was no main effect for group ($F_{1, 7} = 4.171$, $P = 0.08$). The % increase in HR relative to baseline was not significantly different on exercise and on recovery in CD compared to HV. There was a main effect of exercise ($F_{1,7}$, 338.17, $P < 0.001$). There was a significant increase in HR on exercise at 50% $VO_2$ peak relative to baseline in both HV and CD groups (56.12 ± 8.9 vs 66.9 ± 14.45). On recovery, the % change in HR from baseline was -2.8 ± 9.38 vs 11.84 ± 8.34 in HV and CD respectively. There was no interaction effect $F_{1, 7} = 0.383$, $P = 0.555$. Both HV and CD showed a comparable % increase in HR on exercise and a return toward baseline values during recovery from exercise.

% change SV: There was no main effect for group on stroke volume (SV) ($F_{1,7} = 0.340$, $P = 0.578$). SV increased on exercise and returned to baseline values during recovery from exercise in both HV and CD. There was a main effect of time (1, 7, 18.98, $P = 0.003$). The % change in SV relative to baseline values was greater on exercise than on recovery ($P < 0.003$). There was no interaction effect $F_{1,7} = 0.004$, $P = 0.953$). Both HV and CD showed a comparable % increase in SV from baseline on exercise and returned toward baseline during recovery from exercise.

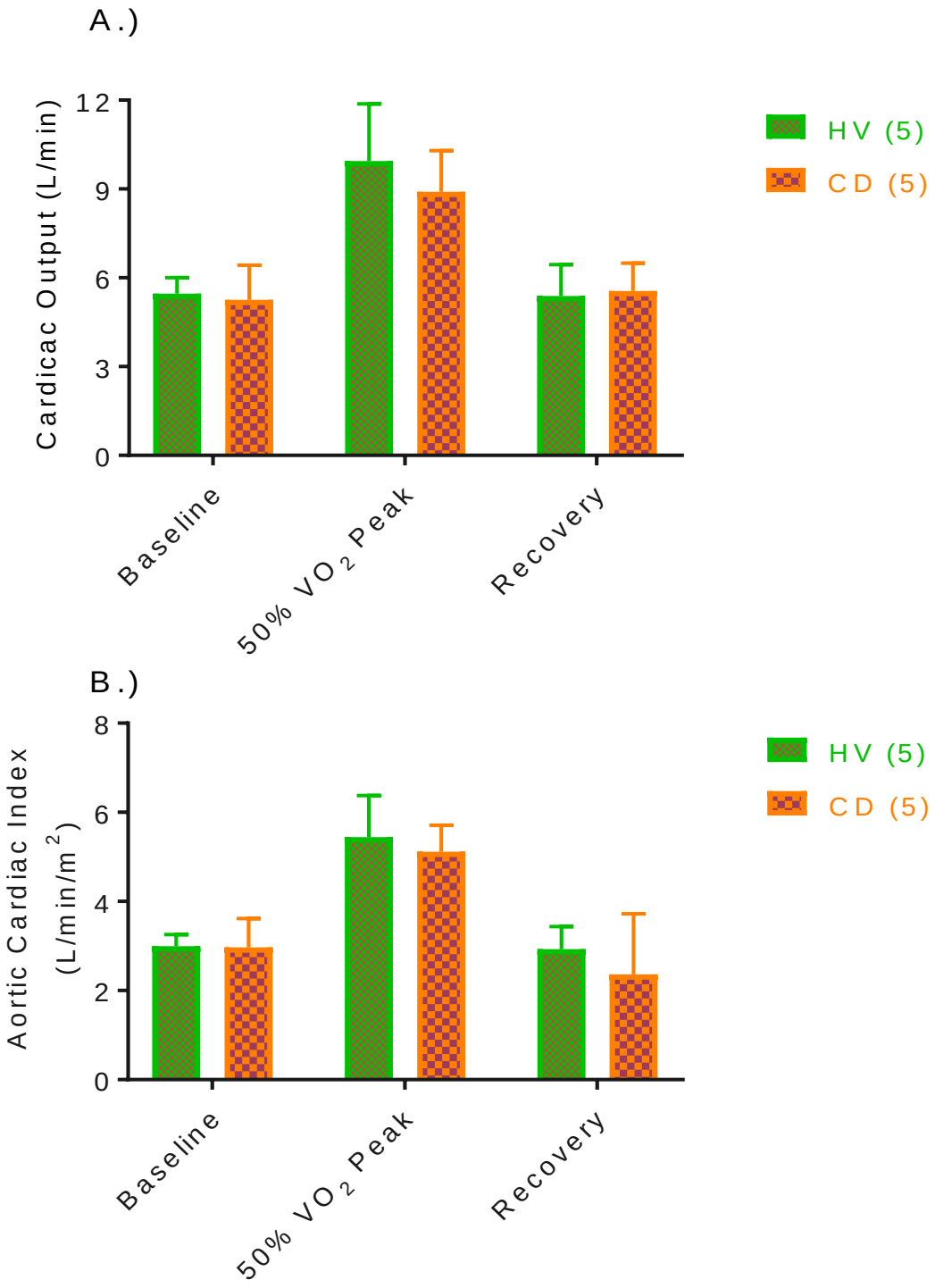


***Figure 6-3*** *A) Cardiac output and B) cardiac index across the exercise task in the HV and CD groups.*

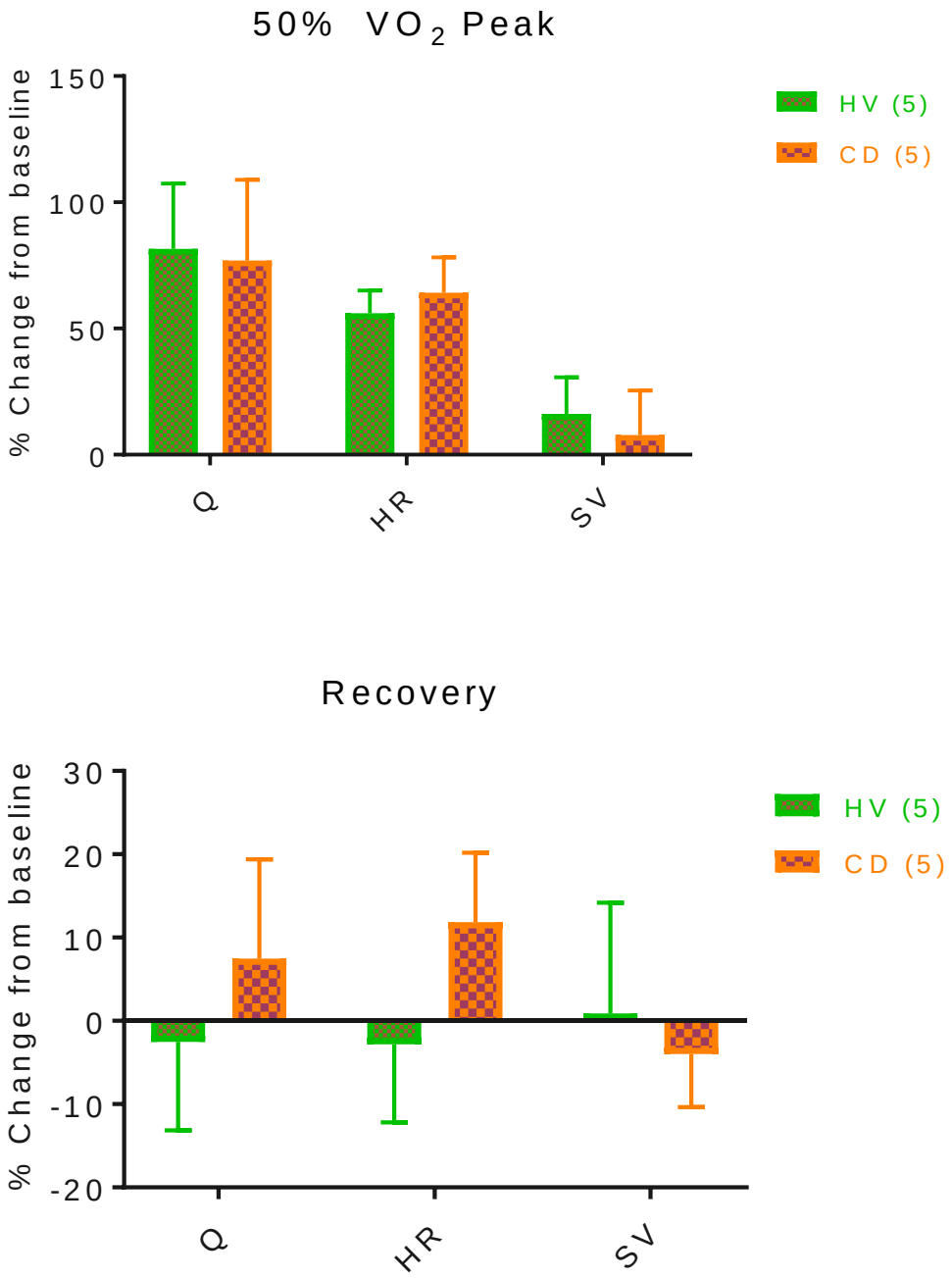


***Figure 6-4.*** *% change in cardiac output and stratification into heart rate and stroke volume changes at 50% VO2 peak and recovery from exercise. Values relative to baseline measurements*

There was a significant positive correlation between $VO_2$ and cardiac output during supine stepping exercise at 50% $VO_2$ peak ($r_5$ = 0.87, 95% CI 0.54 to 0.97, *P* = 0.0009).

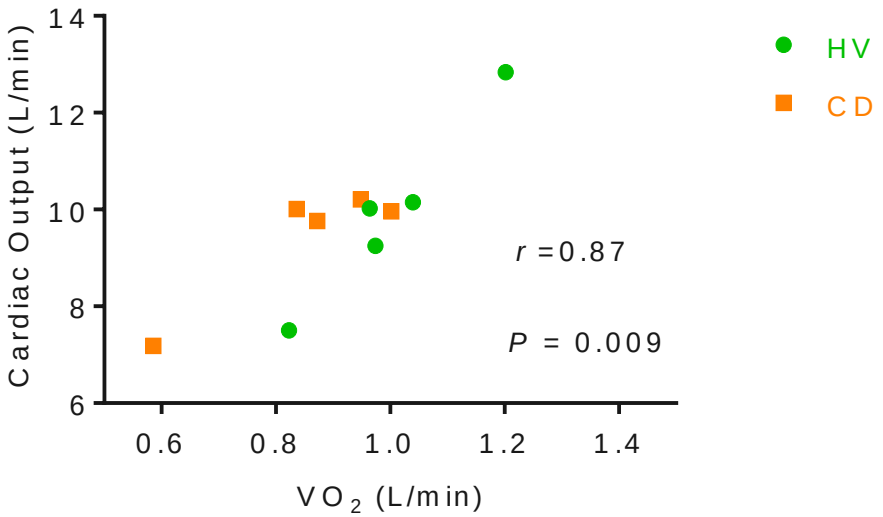


***Figure 6-5.*** *Relationship between $VO_2$ and cardiac output during supine stepping exercise at 50% $VO_2$ peak. (N=5 HV and 5 CD). Note that reduced sample size reflects AO Flow data loss (see figure 6-1 and 6-3).*

### 6.5.5 Respiratory Physiology

The mean minute ventilation (VE) for the Crohn's disease group during supine exercise at 50% $VO_2$ peak (22.6 ± 5.3L/min) was lower ($t_{16}$ = 2.44, *P* =0.027) than the mean of the healthy control group (27.9 ± 3.4). The 95% CI for the mean of the difference was from 0.69 to 9.8 L/min.

The mean expired $CO_2$ ($VCO_2$) for the Crohn's disease group during supine exercise at 50% $VO_2$ peak (11.04 ± 2.4 ml/kg/min) was significantly lower ($t_{16}$ = 2.18, *P* =0.045) than the mean

of the healthy control group (13.23 ± 1.7 ml/kg/min). The 95% CI for the mean of the difference was from 0.05 to 4.32 ml/kg/min.

There were no significant differences in $VO_2$ between healthy controls and Crohn's disease (14.04 ± 2.4 vs 12.3 ± 2.1, $P$ = 0.12) during supine exercise at 50% $VO_2$ peak. Both Arterialised-venous $PCO_2$ (5.34 ± 0.24 vs 5.16 ± 0.59 kPa, $P$ = 0.54) and petCO2 (41.03 ± 1.55 vs 39.18 ± 3.36 mmHg, $P$ = 0.20) were consistent between groups.

| CPET Parameter | **HV** | **CD** | ***P* - value** |
|---|---|---|---|
| VE (L/min) | 27.9 ± 3.4 | 22.6 ± 5.3 | 0.027***** |
| $VCO_2$ (ml/kg/min) | 13.23 ± 1.7 | 11.04 ± 2.4 | **0.045*** |
| $VO_2$ (ml/kg/min) | 14.04 ± 2.4 | 12.3 ± 2.1 | **0.12** |
| $PCO_2$ kPa | 5.34 ± 0.24 | 5.16 ± 0.59 | 0.54 |
| $PetCO_2$ mmHg | 41.03 ± 1.55 | 39.18 ± 3.36 | 0.20 |

***Table 6-4**. CPET parameters and $PCO_2$ from arterial blood gas analysis at 50% $VO_2$ peak. * = $P < 0.05$.*

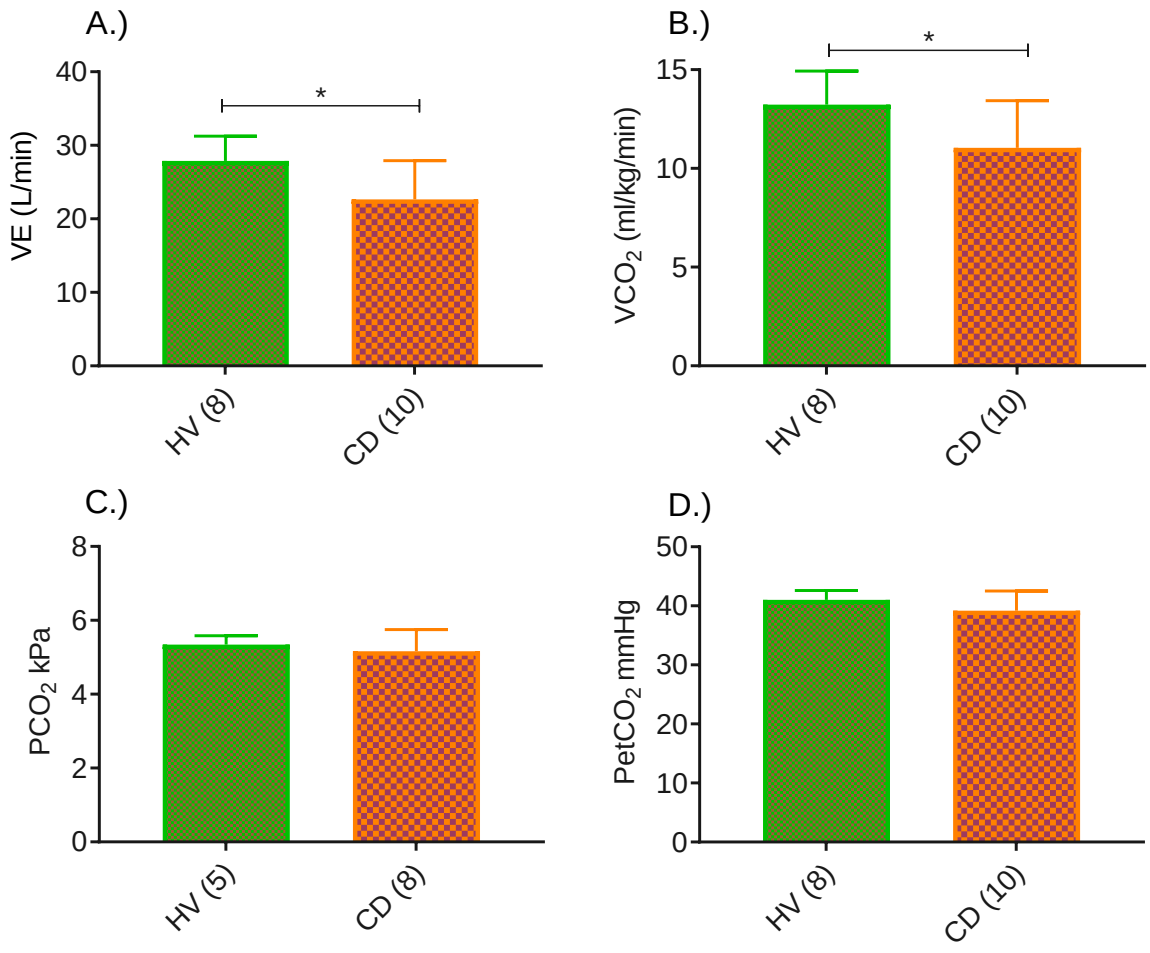


***Figure 6-5**. Respiratory physiology plots, minute ventilation, expired $CO_2$, arterial $CO_2$ content and end-tidal $CO_2$. * = $P < 0.05$.*

### 6.5.6 Cerebral blood flow

There was a significant main effect for group ($F_{1, 17} = 4.78$, $P = 0.043$) with global CBF being higher in the HV group compared to the CD group across the task (Fig. 3A). There was a significant effect of time ($F_{2, 34} = 4.75$, $P = 0.015$), with a trend for a CBF increase on exercise relative to baseline ($P$=0.076), and a significant reduction from exercise to end recovery ($P$ = 0.05). There were no differences between baseline and end recovery CBF values ($P$ = 1.0). The interaction effect did not reach statistical significance ($F_{2, 34} = 3.14$, $P = 0.056$), HV

demonstrated an increase in CBF on exercise which returned to baseline levels during recovery, whereas there was no response in CBF across the three time points in the CD group.

On correcting CBF for grey matter volume, there remained a significant main effect for group ($F_{1,17} = 6.156$, $P = 0.024$), where gmCBF was higher across the task in the HV group compared to the CD group (Fig. 3B). There was a significant effect for time ($F_{2, 34} = 4.589$ $P = 0.017$). gmCBF did not significantly increase on exercise relative to baseline ($P = 0.089$) although there was a significant decrease during recovery relative to during exercise ($P= 0.043$). There was no difference between baseline and end recovery gmCBF values ($P = 1.0$). The interaction effect did not reach statistical significance, ($F_{2\ 34} = 3.221$, $P = 0.052$). The HV group showed an increase in gmCBF on exercise and a return to baseline during recovery, whereas there was no response in gmCBF across the three time points in the CD group.

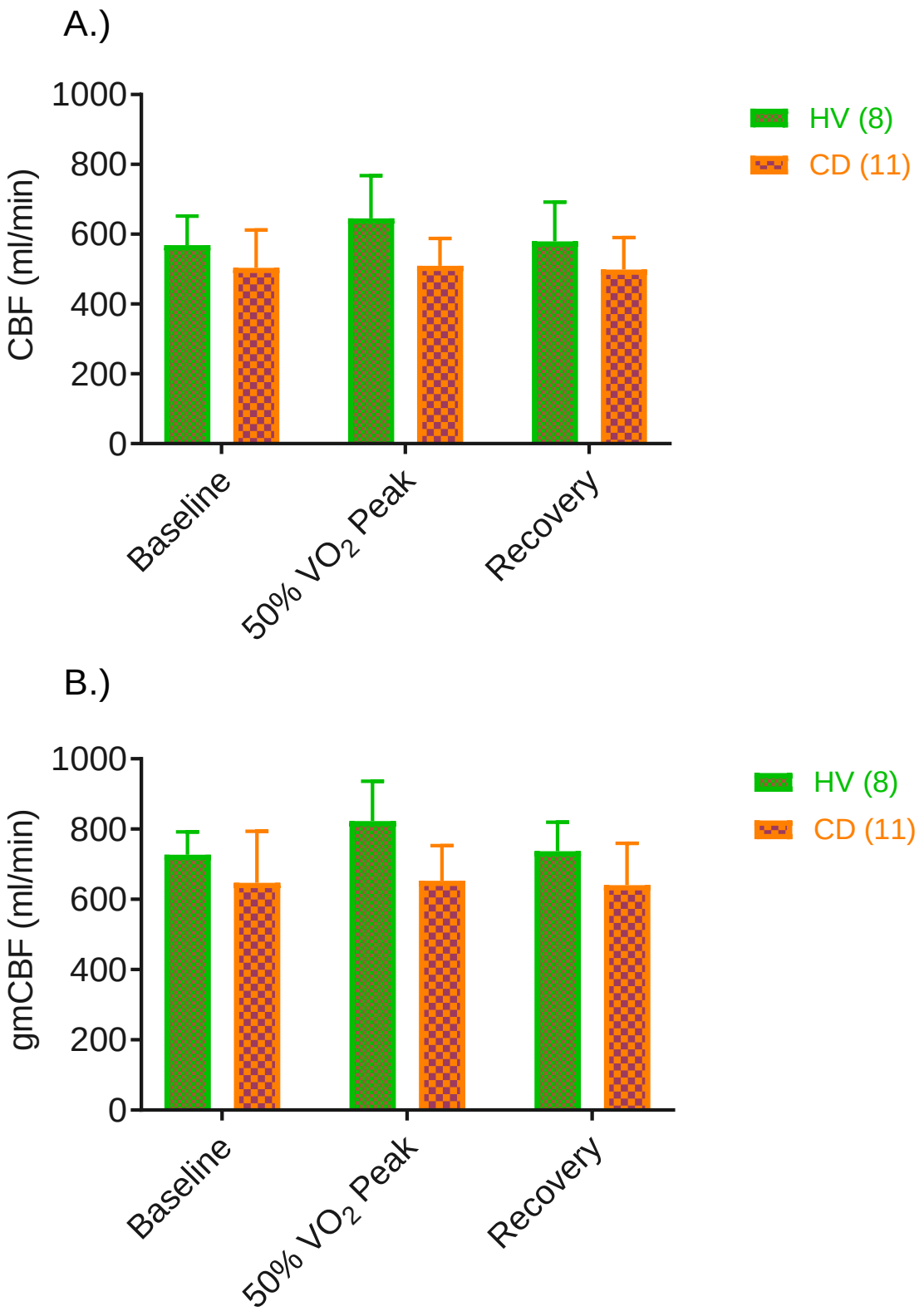


***Figure 6-6*** *A.) Global cerebral blood flow and B.) Grey matter corrected CBF in HV vs CD.*

### 6.5.7 Brain Oxygen metabolism

There was no significant main effect of oxygen extraction fraction (OEF) between groups ($F_{1, 16} = 2.041$, $P = 0.172$). Brain OEF across the exercise task was similar between the HV and CD groups (Fig. 4). There was no significant main effect for time ($F_{2, 32} = 2.91$, $P = 0.069$), OEF decreased non-significantly on exercise challenge relative to baseline measurements ($P$=0.249) and returned to baseline during recovery ($P = 1.0$). There was no significant interaction effect ($F_{2, 32} = 2.03$, $P = 0.148$) with both HV and CD groups showing comparable decreases in OEF on exercise and a return to baseline on recovery.

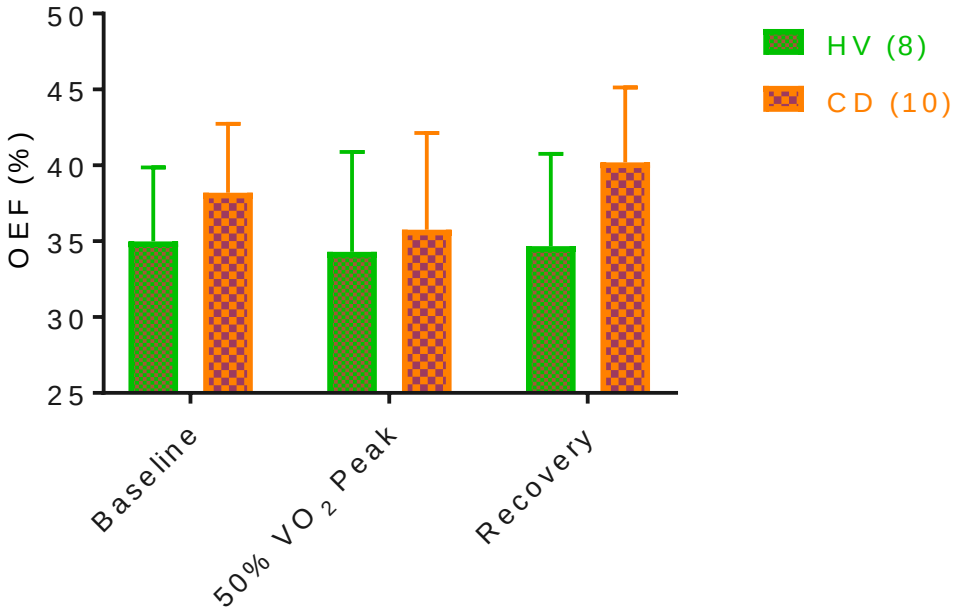


***Figure 6-7****. Percentage oxygen extraction fraction (OEF %) across the exercise task in the HV and CD groups. Data analysed by two-way mixed design ANOVA.*

There was no significant main effect between groups in mean $gmCMRO_2$ ($F_{1, 16} = 0.088$, $P = 0.771$), with measures comparable in the HV and CD groups. There was no main effect for time, $F_{2, 32} = 0.154$, $P = 0.858$), mean $gmCMRO_2$ was comparable across baseline, exercise

and recovery time points ($P$=1.0). There was a significant interaction effect $F_{2, 32} = 5.705$, $P = 0.008$) with the HV group displaying a non-significant increase in $gmCMRO_2$ on exercise whilst the CD group showed a non-significant reduction. Both groups returned to baseline on recovery.

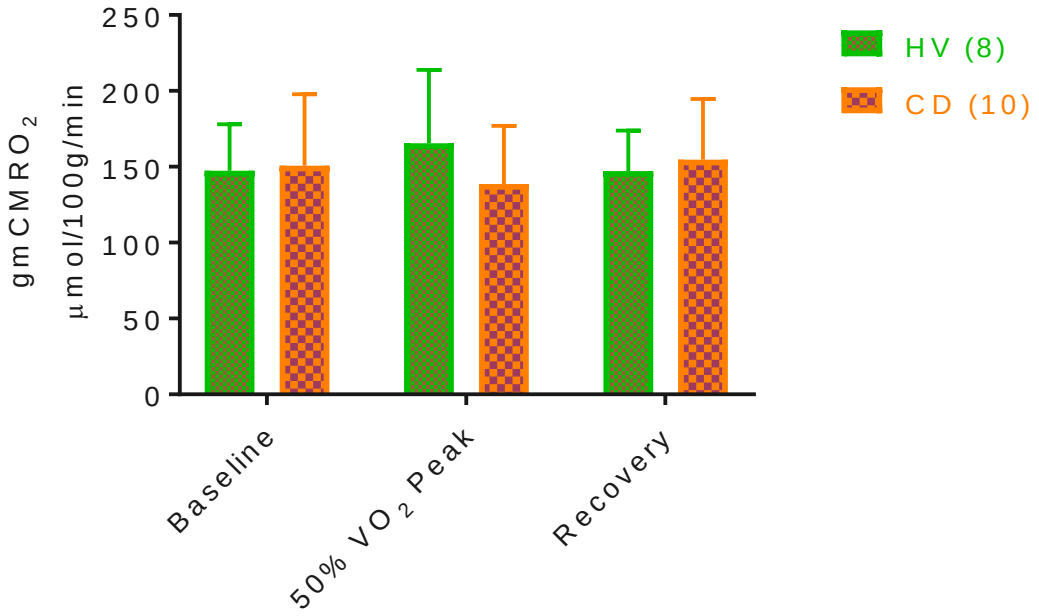


***Figure 6-8**. gmCMRO2 across the exercise task in HV vs CD. Data analysed by two-way mixed design ANOVA*

### 6.5.7 Percentage change in central measures relative to baseline

**Cerebral blood flow and oxygen metabolism**

There was no significant group effect on percentage change in CBF ($F_{1, 17} = 1.079$, $P = 0.313$), the mean percentage change in CBF on exercise and recovery relative to baseline values was similar between groups. There was a main effect for time ($F_{1, 17} = 6.789$, $P = 0.018$), the percentage change in CBF relative to baseline was significantly greater on exercise than end recovery ($P = 0.018$). There was no significant interaction between groups ($F_{1, 17} = 2.41$, $P = 0.139$), both groups demonstrated an increase in CBF on exercise, which returned to baseline on recovery.

For the percentage change in OEF, there was no significant group effect ($F_{1,16} = 0.028$, $P = 0.870$). There was a main effect for time $F_{1, 16} = 5.312$, $P = 0.035$), with the percentage change in OEF from baseline being significantly lower on exercise than end recovery ($P = 0.035$). There was no significant interaction ($F_{1, 16} = 2.649$, $P = 0.123$), with OEF being reduced by a similar percentage in both groups on exercise and which returning toward baseline values during recovery.

There was no significant group effect ($F_{1,16} = 2.117$, $P = 0.165$) in the mean percentage change in $gmCMRO_2$ on exercise and end recovery. There was no main effect of time ($F_{1, 16} = 0.039$, $P = 0.846$), the mean percentage change in $gmCMRO_2$ on exercise and recovery were not significantly different ($P = 0.846$). There was a significant interaction effect ($F_{1, 16} = 6.416$, $P = 0.022$), with the mean % change in $gmCMRO_2$ increasing on exercise in the HV group whereas the CD group showed a mean decrease relative to baseline measurements. Both groups demonstrated a small % increase in mean $gmCMRO_2$ on recovery from exercise relative to baseline.

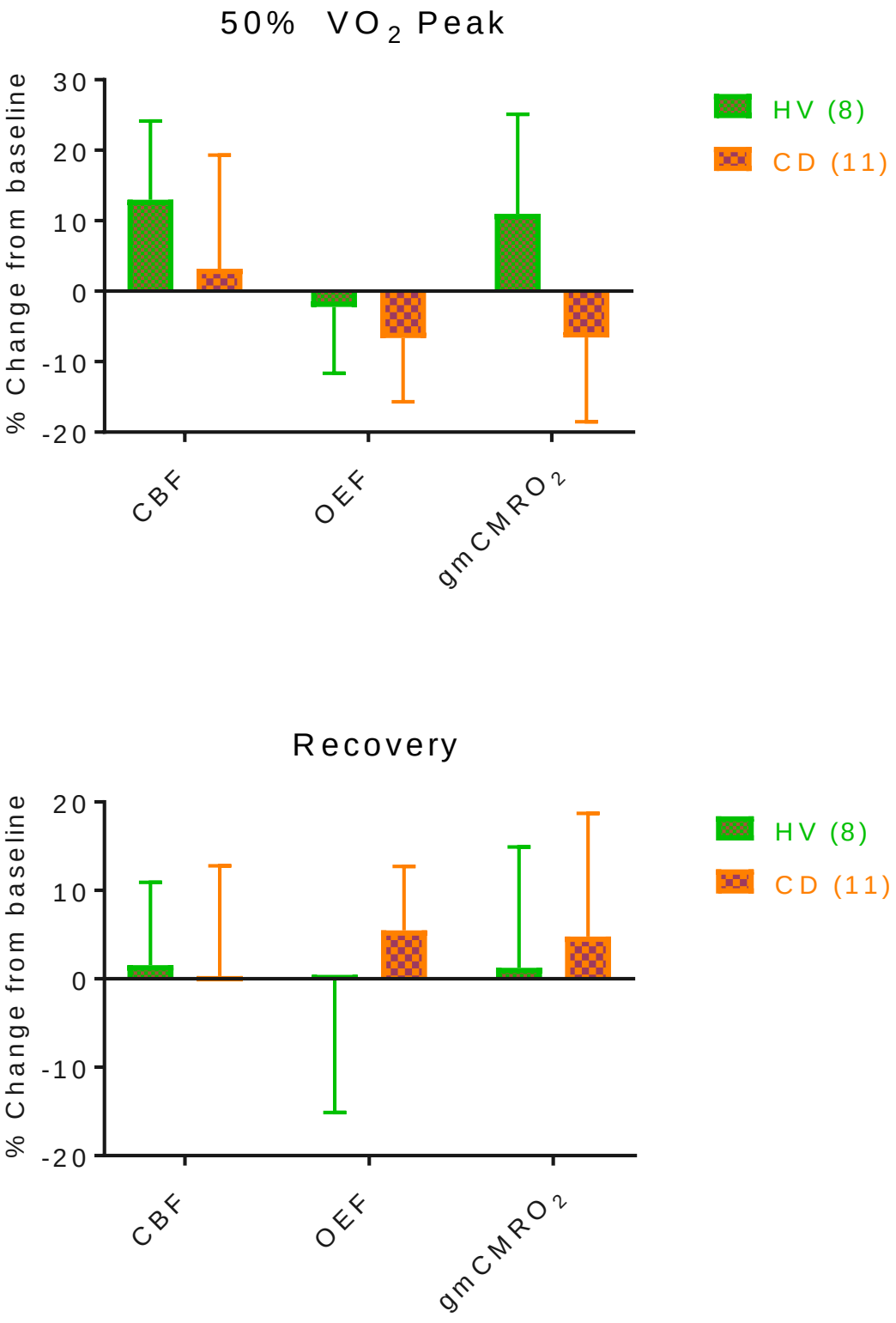


***Figure 6-9.*** *Percentage change from baseline on exercise and recovery in cerebral blood flow, oxygen extraction fraction and grey matter corrected $CMRO_2$ between the HV and CD group. Data analysed by Two-way mixed design ANOVA.*

## 6.6 Discussion

### 6.6.1 Central Measures

In this study, we did not find any evidence of whole-body deconditioning in our quiescent CD patients with a range of self-reported fatigue perception scores. Supine $VO_2$ peak, minute ventilation and expired $CO_2$ volumes were comparable between HV and CD groups, as was arterialised venous blood lactate concentrations (Table 6-2). The exercise modality of supine stepping using the Cardiostepper at 50% of $VO_{2max}$, chosen to reduce motion artefact, recruits a comparatively small amount of muscle mass relative to supine cycling and other traditional modes of exercise. For example, peak $VO_2$ values measured at the University of Nottingham during supine cycling were significantly greater than that of observed using the Cardiostepper. The recruitment of lower muscle mass during supine stepping exercise likely explains the maintenance of $VO_2$ peak reported between our HV and CD groups, which is in contrast to the available literature. Considering the data presented in the later chapter 7 showing muscle metabolic decline, it is reasonable to postulate that performing a traditional CPET (e.g. using a cycle ergometer) may have revealed performance deficits in this cohort similar to that widely reported in the literature [2, 3].

The cardiovascular responses to the exercise challenge were no different between healthy volunteers and Crohn's disease patients. Both cardiac output and aortic cardiac index were consistent across the exercise task in both groups. Relative to baseline, a significant elevation was observed in both groups during supine stepping exercise at 50% $VO_2$ peak, but the response was matched between groups. Consistently, $VO_2$ remained the same in both groups. On exercise recovery, cardiac output returned to baseline levels in both groups. These data

provide evidence of maintenance of cardiovascular function in Crohn's disease patient under both resting conditions and during continuous, low-intensity exercise.

### 6.6.2 Respiratory data

At the respiratory level, both minute ventilation (VE) and expired CO2 ($VCO_2$) were significantly less in Crohn's disease relative to healthy volunteers during supine stepping exercise at 50% $VO_2$ peak. This was observed alongside a blunted gmCBF response to exercise in the Crohn's disease patients, which did not increase from baseline levels and was significantly lower during the exercise challenge relative to healthy volunteers. Despite the elevation of gmCBF on exercise in the healthy volunteers, there were no alterations in the percentage oxygen extraction fraction and cerebral metabolic rate of oxygen across the exercise task in both groups. Thus, brain oxygen metabolism remained unchanged, with no between group differences, despite an increase in CBF in the healthy volunteers.

Initially, it appeared that the reduced VE and $VCO_2$ in Crohn's disease patients could simply reflect an increased metabolic demand in the healthy volunteers. It was suggested that the healthy volunteers may have been exercising above the ventilation inflection point which would account for the elevation in VE and $VCO_2$ and gmCBF relative to the Crohn's disease patients. Despite attempts within the protocol design to negate this (standardised relative intensity at 50% $VO_2$ peak).

To test this hypothesis, mean VE data obtained during incremental exercise testing was plotted as a function of workload between HV and CD groups with the corresponding workload utilised during within-bore exercise were plotted on the X axis. These data provided evidence that the workloads corresponding to 50% $VO_2$ peak established during incremental exercise testing, were in fact below the ventilation inflection point in both groups (Figure 6-10). This

confirmed that the prescribed relative exercise intensity had been adequately maintained and matched between both groups. Both healthy volunteers and Crohn's disease patients were exercising below the ventilator inflection point during the exercise challenge as originally intended. Thus, this hypothesis cannot explain the lower VE, $VCO_2$ and gmCBF observed in the Crohn's disease patients relative to healthy volunteers.

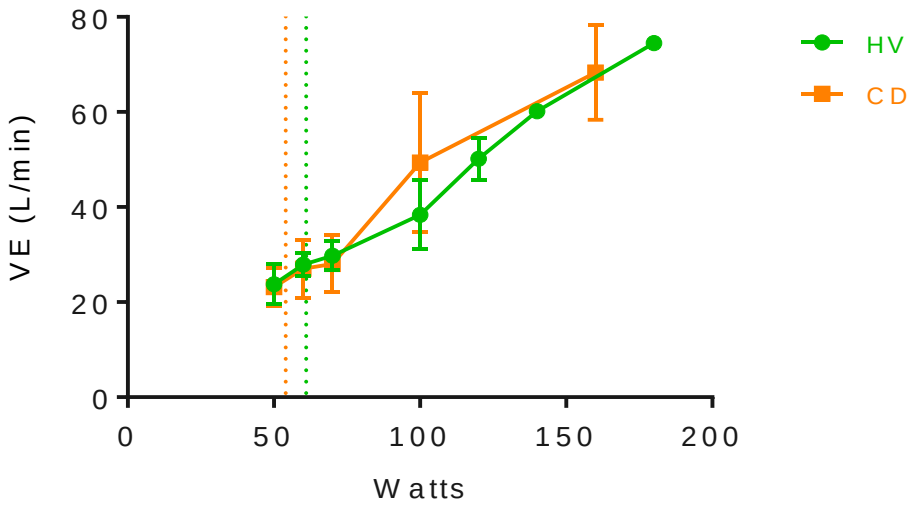


***Figure 6-10**. Minute ventilation (VE) as a function of workload (watts) during supine incremental exercise testing on the Cardiostepper in healthy volunteers and Crohn's disease patients. Dashed vertical lines represent the mean workload corresponding to 50% $VO_2$ peak in both groups.*

It was also considered whether the increase in absolute workload between the healthy volunteers and the Crohn's disease patients (Figure 6-10) created a greater metabolic demand in the healthy controls. Although again this is not consistent with two points. Firstly, the fact that these workloads represent a relative exercise intensity matched between groups. Secondly,

the maintenance of both cardiac output during within-bore exercise and of $VO_2$ during laboratory exercise at the same relative workload, corresponding to 50% $VO_2$ peak.

Another point to consider is establishing $VO_2$ peak. This is known to be difficult to achieve for various reasons relating to the familiarity of exercise, motivation and thus the ability to exercise to volitional exhaustion [18]. This is an important consideration given that we assessed a patient cohort with variable self-reported fatigue symptoms which was higher on average than healthy controls in the general domain. Further peripheral muscle decline (next chapter) and an un-trained healthy control cohort too. The unfamiliarity of all volunteers to supine stepping exercise is a further important consideration. Despite a dedicated familiarisation period, Crohn's disease patients did tend to experience more difficulty during the initial stages of the exercise task and required more verbal encouragement and guidance to maintain the required step frequency and power. Generally, this improved over the course of the test, which is consistent with the reported $R^2$ values from linear regression (Table 6-2) performed between the $VO_2$ and workload data (relative workload calculations). Any subject who could not perform at least 6 incremental workloads or did not obtain an $R^2$ value $\geq 0.95$ was excluded from the study. However, this raises the possibility of whether the patients were able to reach steady-state during the initial workloads, which could have influenced the reduction in VE and $VCO_2$, as these values were calculated from the incremental test data. The resolution of these initial familiarity related issues, during within-bore exercise testing at the second study visit, strongly suggests a learning effect and supports the notion that the initial issue was only related to familiarity to the exercise modality. The maintenance of cardiac output between groups, calculated during the within-bore exercise task and the quality of the incremental laboratory test data (Table 6-2) rules out any Cardiostepper related issues.

A further consideration with respect to exercise modality is that the vast majority of volunteers anecdotally reported excessive peripheral fatigue, localised to the thighs, during and upon cessation of laboratory based incremental exercise testing on the Cardiostepper. Paired with the unfamiliarity to supine exercise, this raises the possibility of whether we were able to establish a true supine $VO_2$ peak. Firstly, it is possible that the Crohn's patients volitionally terminated the incremental exercise test prior to reaching supine $VO_2$ peak due to peripheral fatigue, which would be consistent with the peripheral deconditioning evidenced by the $^{31}P$ MRS data (discussed in chapter 7). RPE values were generally maximal at the point of termination in both groups (Table 6-1), although this likely relates to the reported perception of peripheral fatigue development. Second, had the same issue of peripheral fatigue development occurred in the healthy volunteers, we may also have been unable to establish supine $VO_2$ peak in this cohort. This may account for the maintenance of $VO_2$ peak between the Crohn's disease patients and healthy controls, despite evidence of peripheral muscle deconditioning in our patients and the previous reports of impaired cardiorespiratory fitness in IBD [3]. The volunteers may have prematurely terminated exercise prior to supine $VO_2$ peak due to excessive peripheral fatigue and associated RPE elevations. Thus, potentially masking any genuine alterations in $VO_2$ peak between groups.

Having ruled out any potential mechanisms relating to the differential metabolic demand of exercise, it was also considered whether an alteration in $CO_2$ sensitivity could be responsible for the reduced gmCBF Crohn's disease patients. The Crohn's disease patients performed less habitual physical activity than healthy volunteers did and presented with peripheral muscle deconditioning. A recent study reported no associations between physical activity levels and cerebrovascular function in a cohort of 51 healthy older adults [19]. It has been hypothesised that the reduction in BOLD cerebrovascular reactivity measurements observed in master's athletes relative to age matched sedentary controls is an adaptive response to high levels of

$CO_2$ exposure across the lifespan of these athletes, thus dampening the cerebral vasodilation response to $CO_2$ [20]. Although middle aged endurance trained participants were found to have greater cerebrovascular reactivity relative to sedentary healthy controls ($P$ = 0.052) [21]. Another study demonstrated BOLD cerebrovascular reactivity to be negatively associated with $VO_2$ max in frontal brain regions involved in a Stroop task, whereas $VO_2$ max was positively correlated with cerebrovascular reactivity in the white matter periventricular areas and the postcentral gyrus region [22].

The relationship between physical activity, $VO_2$ max and cerebral $CO_2$ sensitivity is not fully characterised and may vary across brain regions [22]. It is unclear whether an inhibition of $CO_2$ sensitivity contributed to the reduction in CBF in the Crohn's disease patients compared with healthy volunteers. It is also important to acknowledge that quantifying CBF by measurement of blood flow in the internal carotid arteries and basilar artery represent blood flow to the cerebrum, rather than actual global CBF. Future assessment of regional brain perfusion in IBD together with cerebrovascular reactivity will aid elucidating the apparent blunting of CBF on exercise in the Crohn's disease patients in the absence of any alterations in cerebral oxygen metabolism.

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# Chapter 7. Muscle metabolic quality in quiescent Crohn's disease patients.

**Abstract**

To complete the comprehensive physiological phenotyping of quiescent Crohn's Disease patients, peripheral muscle deconditioning was assessed by $^{31}$P MRS. Multiple kinetic parameters relating to PCr recovery were calculated during recovery from exhaustive ischemic contraction.

Calf muscle PCr recovery was significantly slowed in Crohn's disease patients relative to healthy volunteers across all kinetic parameters ($k_{PCr,}$ $V_{PCr}$ , $Q_{max)}$ ($P < 0.05$). Calf muscle volume, percentage fat fraction and maximal isometric strength were no different between groups.

The collective findings from this chapter provide clear evidence of peripheral muscle decline in quiescent Crohn's disease patients with variable levels of self-reported fatigue perception, relative to healthy volunteers. This occurs in the absence of central cardiorespiratory deconditioning measured in chapter 7, muscle atrophy, and strength loss. This evidence of impaired muscle mitochondrial function, corroborates existing data on physical performance deficits in IBD and may be linked to fatigue aetiology.

The data presented within this chapter has been presented at the following international meetings:

1. ***McGing, JJ**, Nicholas, R. Serres, S. Greenhaff, PL. Moran, GW & Francis ST. $^{31}$P MRS and MRI phenotyping of muscle metabolic quality in IBD fatigue.Accepted abstract (poster presentation) ECCO, Vienna, Austria 2022*

2. ***McGing, JJ**, Nicholas, R. Serres, S. Greenhaff, PL. Moran, GW & Francis ST. $^{31}$P MRS and MRI phenotyping of muscle metabolic quality in inflammatory bowel disease fatigue. Abstract (Virtual presentation) ISMRM 2021*

3. ***McGing, J** . Nicholas, R. Serres, S. Greenhaff, P. & Francis, S. (2020). Assessment of peripheral muscle deconditioning using $^{31}$P-MRS during high intensity ischeamic plantar flexion exercise. Accepted Abstract, (Virtual presentation) ISMRM, Sydney 2020.*

## 7.0 Introduction

The rationale for the MR based assessment of deconditioning in IBD was outlined in the introduction to chapter 2. Chapter 2 also provides an in-depth overview of the available data on exercise performance in IBD patients and the commonly reported performance decrements when compared to healthy volunteers.

The comprehensive assessment of body composition, peripheral muscle function (maximum strength and fatiguability) in chapter 5 revealed no differences between quiescent Crohn's disease group relative to age and BMI matched healthy volunteers. Similarly, the use of supine cardiopulmonary exercise testing in tandem with multiple imaging methods to assess the neurometabolic response to a low-intensity supine stepping exercise task in chapter 6 showed no conclusive evidence of central cardiorespiratory deconditioning in the quiescent Crohn's disease patients with varying magnitudes of self-reported fatigue perception relative to the healthy control cohort.

## 7.1 Aims

This chapter aimed to mechanistically assess the role of peripheral muscle deconditioning in the known performance decrements (i.e. premature exercise) fatigue consistently reported in IBD cohorts [1-4] and to thus speculate on the potential contributions to general IBD fatigue aetiology (i.e. self-reported fatigue perception). Calf maximum isometric strength (MVC) was assessed, as was whole-calf muscle volume and percentage fat fraction (calculated from $^{1}H$ MRI). Resting state $^{31}P$ MRS measurements were performed across the calf anatomy to establish baseline $^{31}P$ metabolite concentrations. The rate of PCr recovery was then measured *in-vivo* following high-intensity ischemic plantar flexion exercise to compare muscle metabolic quality in our quiescent CD cohort with a range of self-reported fatigue perception scores to the age and BMI matched HV group.

## 7.2 Methods

All data within this chapter were collected on a 3T Achieva scanner with multinuclear capability.

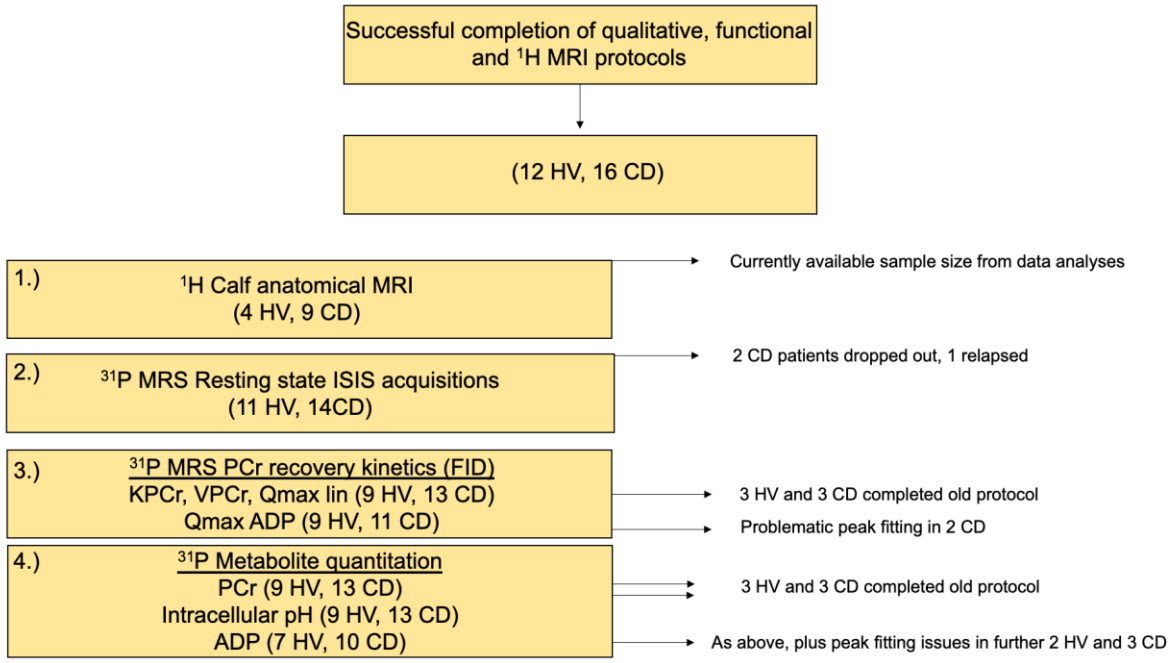


***Figure 7-1.*** *Sample sizes relating to successful completion of experimental assessments followed by stratification of sample sizes for individual MRI and $^{31}P$ MRS acquisitions. Details of metabolic parameters calculated from non-localised.*

### 7.2.1 Peripheral $^{31}P$ MRS measures

Details of the $^{31}P$ MRS methods and analyses are located in chapter 3 & 4.

Cytosolic pH was calculated using the chemical shift difference ($\delta$) between the Pi and PCr peaks:

$$pH = pK + log\left(\frac{\delta 1 - \delta 0}{\delta 0 - \delta 2}\right) \quad (16)$$

where p$K$ = 6.75, $\delta 1$ = 3.27, $\delta 2$ = 5.63.

Free ADP was estimated as:

$$[\text{ADP}] = \left(\frac{[TCr]}{[PCr]} - 1\right)\left(\frac{[ATP]}{(K[H+])}\right) \quad (17)$$

where $K$ = 1.66 *$10^9$ l $mol^{-1}$ and ATP and TCr are assumed to be 8.2 mM and 42.5 mM/ L cell water.

The PCr recovery curve was fit to a mono-exponential function in GraphPad prism:

$$PCr(t) = PCr_{initial} + (PCr_{end} - PCr_{initial})(1 - e^{-kt}) \quad (18)$$

where $t$ is the time from the start of recovery, $PCr_{initial}$ and $PCr_{end}$ is the PCr content at the initial and end of recovery phases.

The post-exercise PCr resynthesis was estimated as:

$$V_{PCr} = k_{PCr} \times \Delta[PCr] \quad (19)$$

where $k_{PCr}$ is the rate constant of PCr resynthesis and $\Delta[PCr]$ is the end recovery PCr concentration minus the end-exercise PCr concentration in mM/L cell water.

$Q_{max}$ was estimated as:

$$Q_{max} = k_{PCr[basal\ PCr]} \quad (20)$$

where $k_{PCr}$ is the rate constant of PCr resynthesis and basal PCr is the resting PCr concentration in mM/L cell water.

$Q_{max\ ADP}$ was estimated as:

$$Q_{max} = V_{PCr}\left(1 + \frac{Km}{[ADP]end}\right) \quad (21)$$

where $V_{PCr}$ is the post-exercise PCr resynthesis and $K_m$ = 30μm/L is the equilibrium constant of the creatine kinase reaction.

### 7.2.2 Statistical analyses

Statistical analyses of the data were performed using IBM SPSS Statistics Version 26. Data were checked for normality by visual inspection of normality plots. Between group comparisons of single dependent variables were carried out by independent samples *t*-test. Between group comparisons of $^{31}$P metabolites across the within-bore exercise task were carried out by a two-way mixed design ANOVA with a Bonferroni correction, to assess the main effect of group (HV vs CD), time (baseline, exercise, recovery) and the combined effects of both factors. Sphericity was assessed by Mauchly's test of sphericity. If the Greenhouse-Geiser epsilon was ≥ 0.75, the Huynh-Feldt corrected value was used to correct the degrees of freedom, else the Greenhouse-Geisser correction was used.

With respect to sample size estimations, post-exercise PCr ½ time is 35s ± 3 in HVs vs 45 ± 4s in COPD patients [5] who are known to be deconditioned and fatigueable. Assuming power of 80% and α=0.05, 4 subjects in each group would be required to show a difference in $k_{PCr}$ between a healthy control group and a fatigueable cohort with chronic disease.

## 7.3 Results

### 7.3.1 Baseline characteristics

Habitual physical activity measured by step count was significantly greater in the healthy volunteers relative to the Crohn's disease patients (9436 ± 3577 vs 5599 ± 2960, $t_{16} = 2.4$, $P = 0.026$). The 95% CI for the mean of the difference was from 482 to 7193.

| Demographics | | | |
|---|---|---|---|
| | HV (N=9) | CD (N=13) | *P* - Value |
| Age | 41 ± 13 | 42 ± 15 | 0.91 |
| BMI | 24.5 ± 3.5 | 23.5 ± 3 | 0.68 |
| **Clinical characteristics** | | | |
| HBI Score | N/A | 2 ± 2 | N/A |
| Disease duration (years) | N/A | 15 ± 13 | N/A |
| Aminosalicylates (%) | N/A | 15.38 | N/A |
| Immunomodulators (%) | N/A | 15.38 | N/A |
| Previous bowel resection (%) | N/A | 23.08 | N/A |
| **Fatigue measurements** | | | |
| MFI General (N= | 8 ± 3 | 13 ± 4 | 0.006 |
| MFI Physical | 9 ± 3 | 11 ± 5 | 0.318 |
| IBDF Scale (S1) | N/A | 7.5 ± 5.3 | N/A |
| IBDF Scale (S2) | N/A | 28.5 ± 20.2 | N/A |

***Table 7-1.*** *Demographic data, clinical characteristics including disease activity (Harvey Bradshaw Index) disease duration, current medications and history of bowel resection expressed as a % of the patient cohort. Multiple Fatigue Inventory 20(MFI-20), IBD Fatigue scale. Depression and anxiety symptoms (HADS), Cognitive function scores (MoCA) and daily step-count measurements in healthy volunteers and Crohn's disease patients.*

### 7.3.2 Muscle $^{1}$H MRI and $^{31}$P MRS

Whole calf muscle volume ($P$ = 0.855) and fat fraction (%) as measured from the mDIXON scan ($P$=0.578) were comparable between the HV and CD groups. Maximum isometric calf strength did not differ between the HV and CD groups whether normalised to appendicular lean mass index ($P$ = 0.283) or whole calf muscle volume ($P$ = 0.235). The mean power output was comparable in the HV and CD groups ($P$ = 0.362), as was the duration of ischemic exercise ($P$ = 0.157).

| | **HV (9)** | **CD (13)** | ***P* value** |
|---|---|---|---|
| Calf volume ($cm^3$) | 710.8 ± 49.9 | 722.7 ± 119.5 | 0.855 |
| Calf fat fraction (%) | 5.2 ± 1.3 | 5.7 ± 2.0 | 0.578 |
| MVC/calf volume | 0.84 ± 0.21 | 0.91 ± 0.23 | 0.59 |
| Duration (s) | 105.6 ± 35.34 | 84.65 ± 38.62 | 0.24 |
| Power (Watts) | 14.4 ± 6.1 | 17.3 ± 6.3 | 0.362 |
| Daily step count | 9436 ± 3577 | 5599 ± 2960 | 0.0275* |

***Table 7-2.*** *HV and CD group comparisons for calf muscle volume and fat fraction (%), calf maximum voluntary contraction (MVC) normalised to appendicular lean mass index and whole muscle volume. Mean power output and exercise duration. P value calculated from independent t-test. Variable sample sizes due to experimental factors (detailed in Figure 7-11).*

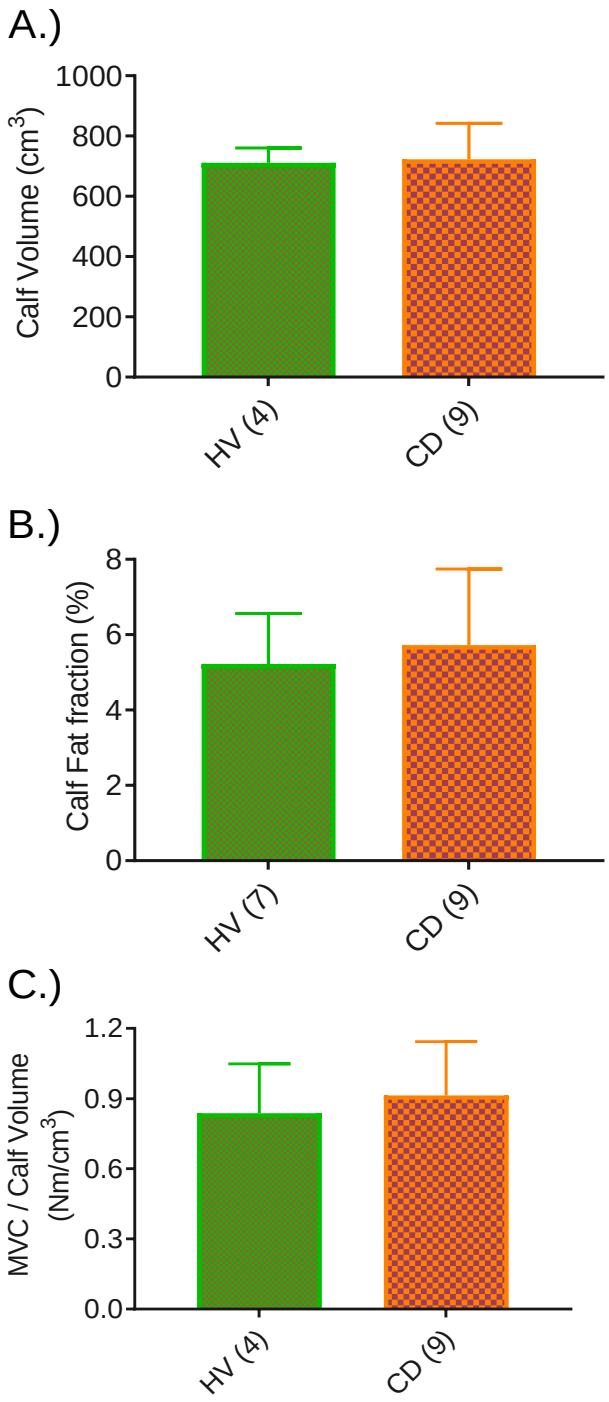


***Figure 7-2.*** *CD vs HV comparisons of calf muscle volume and fat fraction (%), calf maximum voluntary contraction (MVC) normalised to whole muscle volume. Data analysed by independent samples t-test.*

### 7.3.3 Cytosolic pH

There was no significant main effect between groups ($F_{1, 13} = 1.05$, $P = 0.325$) in the estimated muscle pH values across the exercise task (Table 7-3, Fig. 7-3, B). There was a significant main effect for time ($F_{2, 26} = 75.73$, $P < 0.001$), end-exercise muscle pH was significantly lower compared to baseline pH ($P < 0.001$). Muscle pH then significantly increased from end exercise to end recovery ($P < 0.001$), returning to baseline values ($P = 1.00$). There was no significant interaction effect between HV and CD groups ($F_{2, 26} = 0.686$, $P = 0.512$.) Both HV and CD groups showed a significant reduction in muscle pH on exercise, which returned to baseline values at the end recovery period.

### 7.3.4 Muscle PCr concentrations

There was no significant main effect for group ($F_{1, 20} = 1.536$, $P = 0.230$) in calf muscle PCr concentrations (Table 7-3, Fig. 7-3, A). There was a significant main effect for time ($F_{1.834, 36.68} = 337.166$, $P < 0.001$). End-exercise muscle PCr concentration was significantly decreased relative to baseline PCr ($P < 0.001$), muscle PCr then significantly increased from end exercise to end recovery ($P < 0.001$), returning to baseline values ($P = 1.00$). There was no significant interaction effect between HV and CD groups ($F_{1.834, 36.682} = 0.765$, $P = 0.462$). Both HV and CD groups showed a significant reduction in muscle PCr following the exercise task, which returned to baseline values following exercise recovery.

### 7.3.5 Muscle ADP concentrations

There was no significant effect between groups ($F_{1,15} = 0.252$, $P = 0.623$) in calf muscle ADP concentrations (Table 7-3, Fig. 7- 3,C). There was a main effect of exercise ($F_{1.05, 15.72} = 13.58$, $P = 0.002$), end exercise ADP concentrations were increased significantly compared to baseline ($P$=0.009). ADP levels then significantly decreased from end exercise to end recovery ($P$ =

0.004), returning to baseline levels ($P$ = 1.00). There was no significant interaction effect ($F_{1.05\ 15.72}$ =0.027, $P$ = 0.881). Both groups showed comparable elevations in end-exercise ADP concentrations relative to baseline, which returned to baseline at the end of recovery.

### 7.3.6 PCr recovery kinetics

Figure 7-4 shows PCr recovery curves for both the HV and CD group. The mean rate constant of PCr resynthesis ($k_{PCr}$) for the CD group (0.80 ± 0.26 $min^{-1}$) was significantly lower ($t_{20}$ = 2.55, $P$ < 0.019) than that of the HV group (1.07 ± 0.23 $min^{-1}$). The 95% CI for the mean of the difference was from 0.05 to 0.50 $min^{-1}$. (Table 7-4, Fig 7-4 & 5)

The mean post-exercise PCr resynthesis ($V$PCr) for the CD group (17.2 ± 7.1 mM $min^{-1}$) was significantly lower ($t_{20}$ = 2.63, $P$ = 0.016) than for the HV group (25.3 ± 7.2 mM $min^{-1}$). The 95% CI for the mean of the difference was from 1.67 to 14.63 mM $min^{-1}$. (Table 7-4, Fig 7-5, B)

The mean maximal rate of oxidative ATP synthesis ($Q_{max}$ linear model) for the CD group (21.18 ± 8.95 mM $min^{-1}$) was significantly lower ($t_{20}$ = 2.18, $P$ = 0.042) than the mean of the HV group (29.69 ± 9.10 mM $min^{-1}$). The 95% CI for the mean of the difference was from 0.36 to 16.70 mM.$min^{-1}$. (Table 7-4, Fig 7-5, C)

The difference in mean maximal oxidative ATP synthesis ($Q_{max}$ ADP model) in the CD group (23.2 ± 11.2 mM $min^{-1}$) relative to the HV group (32.7 ± 10.7 mM $min^{-1}$) did not reach statistical significance ($t_{18}$ = 1.93, $P$ = 0.07) The 95% CI for the mean of the difference was from -0.86 to 19.85 mM $min^{-1}$ (Table 7-4, Fig 7-5, D)

| $^{31}$P Metabolite dynamics | HV | CD | *P* value |
|---|---|---|---|
| Baseline pH | 7.09 ± 0.10 | 7.18 ± 0.13 | 0.325 |
| End exercise pH | 6.46 ± 0.14 | 6.48 ± 0.12 | |
| End recovery pH | 7.19 ± 0.13 | 7.16 ± 0.20 | |
| Baseline PCr (mM / L cell water) | 27.66 ± 5.59 | 26.21 ± 4.74 | 0.230 |
| End exercise PCr (mM/L cell water) | 5.23 ± 3.08 | 3.80 ± 2.25 | |
| End recovery PCr (mM/L cell water) | 28.91 ± 6.33 | 25.32 ± 5.91 | |
| Baseline ADP (μmol / L cell water) | 33.7 ± 17.9 | 50.68 ± 25.22 | 0.623 |
| End exercise ADP (μmol / L cell water) | 165.0 ± 154.8 | 187.50 ± 151.82 | |
| End recovery ADP (μmol / L cell water) | 40.2 ± 27.7 | 51.75 ± 37.59 | |

*Table 7-3. HV and CD comparisons of $^{31}$P MRS metabolic variables across baseline, high-intensity ischemic contraction through to end recovery. P values reflect the main effect for group (HV vs CD) from a mixed design ANOVA. Sample sizes noted in Fig7-1. Variability due to experimental factors noted in discussion.*

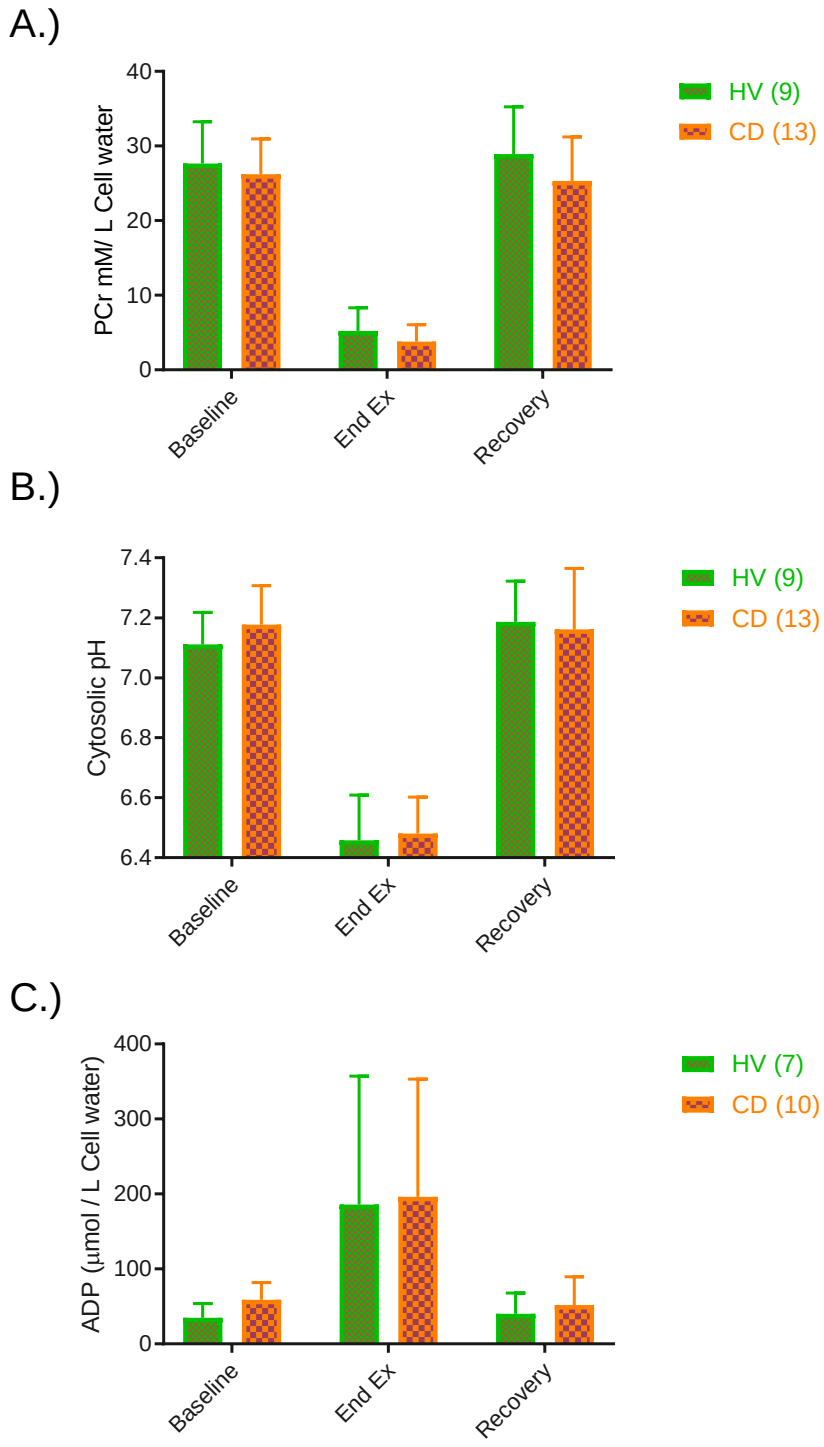


***Figure 7-3.*** *Comparison of HV and CD groups for A) calf muscle PCr concentrations, B) cytosolic pH and C) calf muscle ADP concentrations across the ischemic exercise task. Note that recovery pH measurements are only available for N=6 HV and N=10 CD (see discussion for details).*

| $^{31}$P MRS PCr recovery kinetics | HV (9) | CD (13) | *P* Value |
|---|---|---|---|
| PCr Depletion (%) | 81.22 ± 9.09 | 84.58 ± 8.71 | 0.393 |
| $k_{PCr}$ ($min^{-1}$) | 1.07 ± 0.23 | 0.80 ±0.26 | 0.019* |
| $V_{PCr}$ ($mM.min^{-1}$) | 25.33 ± 7.2 | 17.18 ± 7.1 | 0.016* |
| $Q_{max}$ Linear ($mM.min^{-1}$) | 29.69 ± 9.10 | 21.18 ± 8.95 | 0.042* |
| $Q_{max}$ ADP ($mM.min^{-1}$) | 32.69 ± 10.7 | 23.19 ± 11.2 | 0.07 |

***Table 7-4.*** *Parameters of PCr recovery kinetics including the rate constant ($k_{PCr}$), post-exercise PCr resynthesis ($V_{PCr}$) and maximum rate of oxidative ATP synthesis ($Q_{max}$ linear and ADP models). P values denote between group differences from independent t-test. Sample size listed in brackets.*

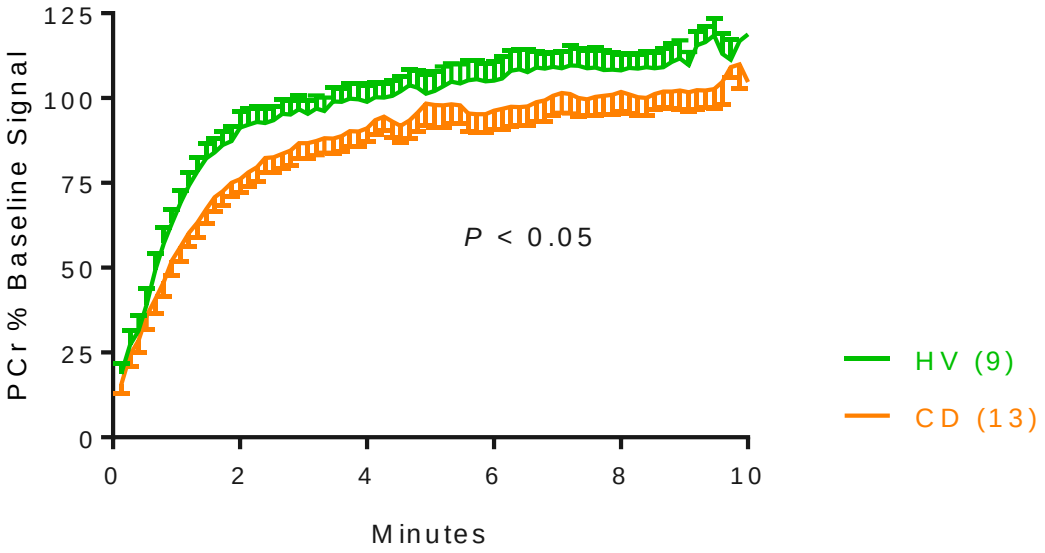


***Figure 7-4**. HV and CD group PCr recovery curves across time, represented relative to baseline PCr signal amplitude. Data presented as mean ± SEM. Error bars plotted in opposite directions to aid data visualisation.*

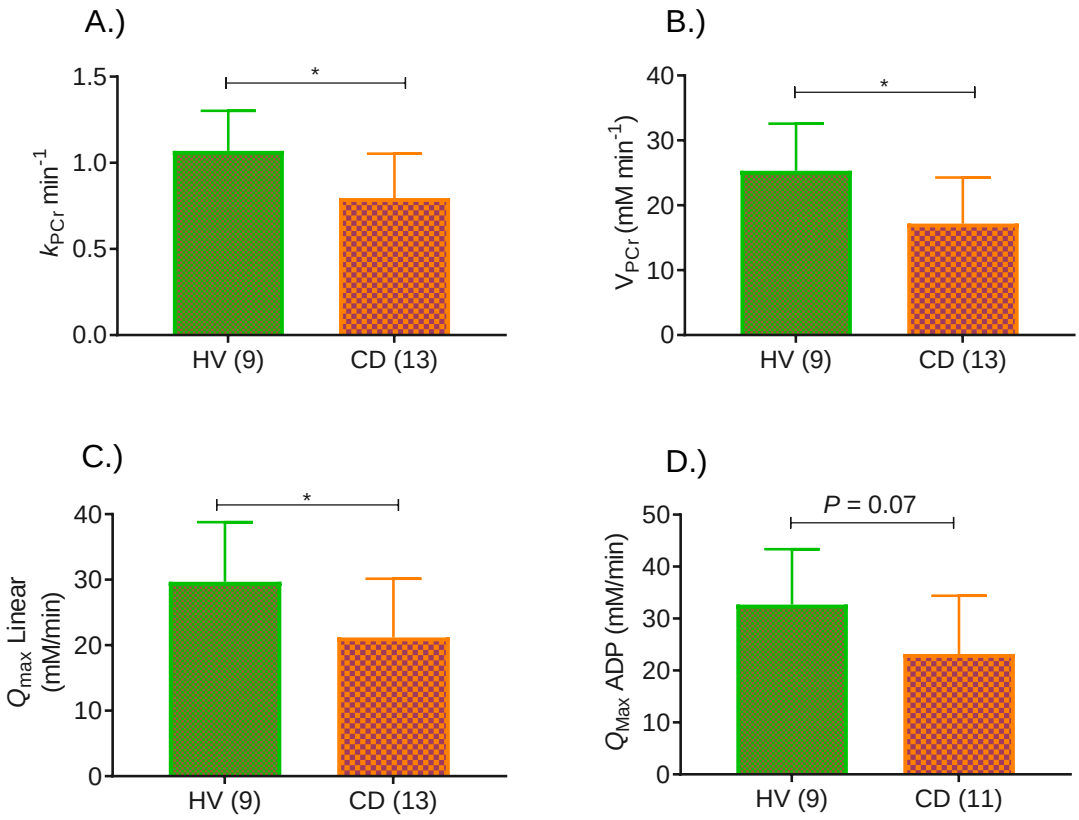


***Figure 7-5.*** *HV and CD comparisons of PCr recovery parameters during recovery from the ischemic exercise task. A.) Rate constant of PCr resynthesis B.) Post-exercise PCr resynthesis. The maximal oxidative ATP synthesis linear (C.) and ADP (D.) models * P < 0.05.*

### 7.3.7 Assessment of the tissue variations in PCr content

There was no significant main effect between groups ($F_{1, 23} = 0.326$, $P = 0.573$) (Table 7-5, Fig 7-6). The reduction in PCr signal amplitude when localising to the soleus and peronei muscle groups relative to the medial gastrocnemius muscle, was consistent between HV and CD. There was a main effect for anatomy / spatial location ($F_{1, 23} = 31.729$, $P < 0.001$), where the mean reduction in PCr signal amplitude in the peronei was greater than the reduction in the soleus, expressed relative to the medial gastrocnemius ( $P < 0.001$). There was no significant interaction between groups, ($F_{1, 23} = 0.001$, $P = 0.981$) the reduction in PCr signal

amplitude measured at the soleus and peronei relative to the medial gastrocnemius was consistent between groups.

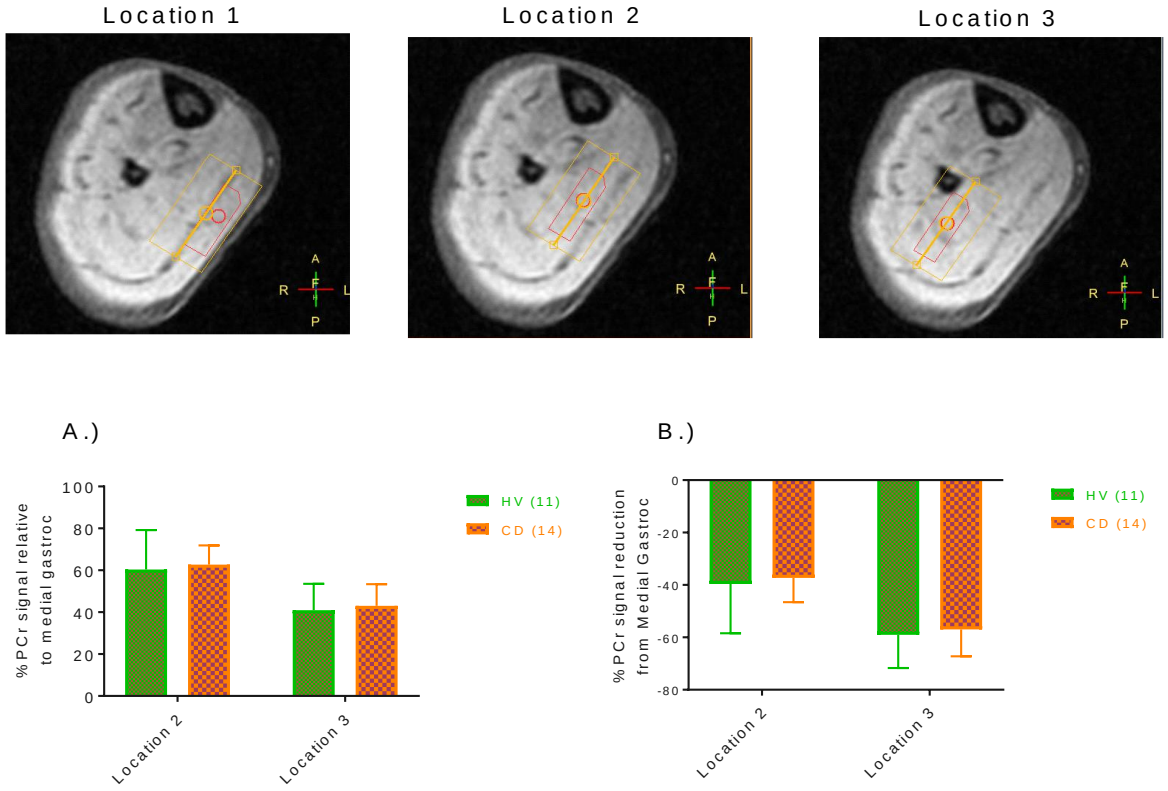


***Figure 7-6.*** *A.) Percentage of PCr signal at location two (soleus) and three (peronei) relative to location one (medial gastrocnemius) in HV and CD. B.) Data A.) Expressed as percentage reduction relative to the PCr signal in the medial gastrocnemius.*

| $^{31}$P MRS ISIS | HV | CD | *P* - Value |
|---|---|---|---|
| Soleus | 39.61 ± 18.81 | 37.32 ± 9.23 | 0.573 |
| Peronei | 59.06 ± 12.61 | 56.94 ± 10.32 | |

***Table 7-5.*** *HV and CD comparisons of PCr signal amplitude (in %) for both soleus and peronei and relative to the medial gastrocnemius and measured using localised $^{31}$P MRS (ISIS).*

### 7.4 Discussion: $^{31}$P MRS Measurements

A slower rate of PCr resynthesis following standardised, high-intensity, ischemic contraction was found in CD patients, relative to an age and BMI matched HV group. This occurred independent of atrophy and strength loss. These data provide evidence of peripheral muscle deconditioning in quiescent CD patients in the absence of muscle mass and strength loss.

These $^{31}$P MRS data are in line with findings of diminished muscle oxidative capacity across a host of chronic diseases presenting with weakness and fatigue including multiple sclerosis (MS) [6], lung transplant recipients [7], renal failure [8] and COPD [9]. These findings provide a metabolic basis for the whole-body deconditioning reported in IBD, such as the progressive decline in peak aerobic workload observed in relation to the extent of disease activity [3]. These data are in agreement with previous data in IBD showing the occurrence of the lactate threshold at a lower $VO_2$ in IBD patients relative to gender specific reference values [2]. In addition, our data provides the first evidence of a decline in muscle quality in quiescent CD patients who self-report variable magnitudes of fatigue perception. This provides a metabolic basis for the premature exercise fatigue frequently reported in IBD [2, 3, 10, 11].

### 7.4.1 Methodological considerations

Our primary outcome variable was $k_{PCr}$ as an index of muscle mitochondrial function, based on the premise of PCr resynthesis relying entirely upon oxidative ATP synthesis [12]. However, it is known that pH acidosis significantly perturbs PCr resynthesis [13, 14] and thus given the results in (Figure 7-3 B, Table 7-3) this may occur here. This may be due to a direct inhibitory effect of pH acidosis on skeletal muscle oxidative phosphorylation [13], although these *in vivo* data are inconsistent with findings from isolated mitochondria, where acidosis has no effect on state three respiration [15]. The effect of low pH on mitochondrial respiration is unclear, with some animal data showing evidence of an inhibitory effect [16] whereas significant acidosis (pH 6.4-6.5) appears to exert relatively little inhibitory effect on mitochondrial respiration in rodent [17] and equine [18] skeletal muscle. There is also reports of increased mitochondrial respiration at low pH [16]. Other potential indirect causes of this acidosis induced slowing of PCr recovery include pH related effects on the creatine kinase equilibrium [19], increased ATP consumption by cellular ion pumps [20] and inhibition of sarcoplasmic reticulum calcium release and reuptake [21]. Thus, under pH acidosis , the general consensus is that various factors can influence PCr recovery, independent of mitochondrial respiration, making $k_{PCr}$ an unreliable marker of muscle mitochondrial function [22]. For this reason, recent recommendations for within-bore exercise experiments recommend limiting contractile intensity such that pH does not reduce by greater than 0.2 units [23].

In light of the significant acidosis induced from our high-intensity ischemic exercise protocol (Figure 7-3, Table 7-3), several further kinetic parameters as indices of muscle mitochondrial function were evaluated. Post-exercise PCr resynthesis ($k_{PCr} \times \Delta[PCr]$) was estimated, to reflect the initial rate of post-exercise oxygen utilisation [24, 25], which was shown to be

independent of pH [22, 26]. Furthermore, the maximal rate of oxidative ATP synthesis was also calculated, according to two commonly used literature models (Linear and ADP models ($Q_{max} = k_{PCr[basal\ PCr]}$ and $Q_{max} = V_{PCr}\ (1 + (K_m/[\text{end-exercise ADP}]))$. The reliability of $Q_{max}$ linear has been disputed given its reliance on $k_{PCr}$ [27]. There is also evidence that $Q_{max}$ ADP is only independent of pH when calculated using the measured $V_{PCr}$ (i.e. the direct measurement of $V_{PCr}$ across the initial post-exercise time points d[PCr]/d$t$) [28], despite the two methods being related in both healthy controls ($r$=0.753) and patient cohorts ($r$ = 0.646) [29]. Conversely, two experiments have demonstrated that estimated $V_{PCr}$ and $Q_{maxADP}$ are independent of cytosolic pH [22, 26] and this parameter remains commonly used [30].

From a metabolic perspective, comparable $V_{PCr}$ and $Q_{max}$ ADP values have been observed in the literature between exercise protocols evoking significant differences in end-exercise pH using measured VPCr [28]. Similarly, estimated $V_{PCr}$ and $Q_{max}$ values were not significantly different despite contrasting end exercise pH values following a low and high frequency exercise protocol (6.80 ± 0.15 in low frequency vs 6.45 ± 0.26 high frequency) resulting in a significant reduction in $k_{PCr}$ between the low and high frequency group (1.3 ± 0.5 vs 0.9 ± 0.5 $min^{-1}$).

Regardless of methodological considerations pertaining to the calculation of these metabolic variables, these data strongly suggest that the post-exercise PCr resynthesis, reflecting post-exercise oxygen utilisation and oxidative phosphorylation [22, 26, 28] is independent of end-exercise metabolic conditions. Therefore, by using $V_{PCr}$ , the potential confounding effects of pH acidosis on the differences in proton efflux rates and thus pH recovery between our groups can be ignored, as they appear to predominate in the later stages of the biphasic PCr recovery [31].

Given that proton efflux and pH recovery are related to PCr recovery during pH acidosis [27], the estimated proton efflux rate and pH recovery across the entire recovery period (~10 minutes) could have been estimated to establish any retarding effect on PCr recovery across this period. However, this depends heavily on having sufficient SNR to be able to accurately fit Pi peaks. Our non-localised $^{31}$P acquisition collected with a relatively large surface coil and intense contractile demand resulted in partial volume effects and in some cases Pi peak splitting [32]. This complicates Pi fitting during the recovery phase with a sufficient temporal resolution to reliably quantity pH recovery. As such, cytosolic pH estimations are limited by block averaging of non-localised $^{31}$P spectra under constant physiological conditions for baseline, end-exercise and recovery periods (see Chapter 3 & 4). In some subjects, end-recovery pH estimations were also problematic due to the well-documented loss of the Pi peak late in the end recovery period. This has been attributed to both sequestering of Pi into the mitochondria [33] and Pi being trapped within the glycogenolytic pathway [34]. This occurred in 6 subjects and accounts for the reduced sample size in end-recovery pH calculations (Fig 7-3 & table 7-3).

A limitation of this work is the use of a non-localised $^{31}$P MRS acquisition scheme. The rationale for this was outlined in detail in the developmental experiments undertaken for this work, and is due to the movement occurring in the occlusion and contraction protocol (Chapter 3). It is known that non-localised acquisitions and the high contractile intensity utilised here, complicate pre-processing of $^{31}$P MRS spectra due to aforementioned partial volume effects. This results from the inclusion of tissue with heterogeneous metabolic characteristics. Partial volume effects include increased line widths [35] and contamination of peaks [23]. Pi peak splitting is also common, particularly at high contractile intensities [32] which is believed to reflect signal differences in oxidative and glycolytic fibres [32].

Implementing a non-localised approach means that the volume of interest is determined by the sensitivity of the $^{31}$P surface coil. The use of a non-localised sequence is therefore based on the assumption that only the target muscle of investigation significantly contributes to the acquired metabolic data. Furthermore, given we have compared HV and CD groups, we assume that the volume of exercising muscle in the $B_1$-field of the coil is matched across groups. This has the potential to skew data, as subjects with larger muscle volumes over the $^{31}$P surface coil and thus greater SNR will demonstrate elevated PCr consumption, which is highly correlated with the kinetic parameters used to quantify PCr recovery (e.g. $k_{PCr}$, $t_{PCr}$, $V_{PCr}$). Concomitantly, increased subcutaneous fat content can increase the distance between the surface coil and the target muscle, thus reducing SNR. The data are also likely to include signal contamination from "non-target" muscle within the sensitive volume of the coil (i.e. soleus contribution to PCr kinetics during plantar flexion when targeting the medial gastrocnemius [36]). This is an important consideration given $^{31}$P metabolism and muscle oxidative capacity is known to vary significantly between muscles [37] and also along the length of individual muscles [30, 38].

Given the potential for signal contamination, prior to our exercise task we performed three localised $^{31}$P MRS ISIS acquisitions at the medial gastrocnemius, soleus and peronei muscles in each volunteer. This enabled us to quantify the spatial variations in PCr content (or signal) across the calf anatomy and give an indication on the potential for signal contamination. These data showed significant PCr content across the calf anatomy, even into the deeper fibres. However, the percentage PCr signal in the soleus and peronei muscle groups relative to the medial gastrocnemius, were consistent between the CD and HV groups. Furthermore, in the HV and CD groups, analysis of $^{1}$H mDIXON scans showed consistent whole muscle volume and percentage of fat fraction between groups (Figure 7-2 & Table 7-2) which is consistent with the maintenance of lean tissue mass and isometric strength between the HV and CD groups. Thus, these data support the assumption that the volume of exercising muscle within

the $B_1$ field of the coil was consistent between the HV and CD groups. However, we cannot exclude the possibility of fibre type discrepancies within this volume of muscle, given the potential for both fibre type transitions or the preferential atrophy of specific fibre types. Alterations in myosin heavy chain expression have been reported in gastrocnemius biopsy's of active CD patients. [39]. In this study, the resulting PCr recovery curves likely representative a weighted sum of PCr recovery rates across various muscle fibres with varying mitochondrial and PCr content. However, it is reasonable to expect that these recovery curves are heavily weighted to the medial gastrocnemius. This is due to its spatial location in relation to the $^{31}$P surface coil and that the high-intensity contractile stimulus would theoretically, maximally recruit this muscle group. The $^{31}$P MRS estimates of PCr reported in the literature tend to be higher than muscle biopsy methods $by \sim 20\%$ which is hypothesised to be due to net PCr breakdown via CK during the freezing process [40]. Despite this, our PCr concentrations were close to those measured by muscle biopsy (Our HV group 27.7 ± 5.6 mM, literature range 27-30mM / L cell water [40]) and individual PCr concentrations were within the literature range reported for uncalibrated $^{31}$P MRS [40].

### 7.5 Conclusion

Encouragingly, all our calculated $^{31}$P kinetic parameters demonstrated consistent reductions in the CD group relative to the HV group. Our $Q_{max}$ ADP data did not reach statistical significance ($P$ = 0.07), this is likely related to a lower sample size as opposed to any physiological discrepancy. The lower sample size was the result of not being able to calculate an accurate end-exercise pH and thus end-exercise ADP concentration in 6 volunteers. Our kinetic parameters (Table 7-4) are comparable to reported literature values in terms of both variability [22] and absolute values [22, 25, 26, 28, 41]. Paired with the additional finding that cytosolic pH (along with all $^{31}$P metabolites) returned to baseline following recovery from exercise (~10

minutes), of which there were no initial between group differences, these data strongly point to a loss of mitochondrial mass in quiescent CD patients (i.e. peripheral muscle deconditioning) as opposed to any inherent mitochondrial abnormality. Inherent defects in mitochondrial function and altered energetic state can result in altered $^{31}$P MRS metabolites at rest such as a reduced ratio of PCr/Pi as observed in myopathy [42]. Other muscle related abnormalities present as an increased resting pH, as observed in dystrophic muscle [43]. We did not observe any such abnormalities in under resting conditions, which can be considered consistent with the maintenance of muscle mass, strength in our CD cohort with well controlled disease. Our evidence of reduced muscle mitochondrial function in the CD group relative to the HV group only became apparent on exercise challenge. This is congruent with data on whole-body deconditioning in IBD [2, 3, 10, 44] and the reduction in daily habitual physical activity across the lifespan [45, 46], which was also present in our cohort.

Collectively, available data [15, 22, 26] indicate that any inhibitory effect of acidosis appears specific to the slower secondary phase of the biphasic PCr recovery [31]. Thus, the kinetic parameters specific to initial PCr recovery can confidently infer on muscle mitochondrial function in the presence of end-exercise acidosis. By using limb occlusion, we have created a closed metabolic compartment such that end-exercise PCr, ADP and cytosolic pH were tightly matched at the point of re-instating limb blood flow and to maximally stimulate mitochondrial respiration. This scenario is not easily achievable during submaximal intensities used to limit acidosis [23] as $^{31}$P metabolism and pH changes are highly variable between subjects [47, 48] and muscle [37]. A variable end-exercise metabolic state would itself complicate the inference of muscle mitochondrial function from PCr recovery kinetics. For example, a slowed PCr recovery in Cystic Fibrosis (CF) patients relative to controls (Halftime of PCr recovery of 27.2 ± 11.7 in CF vs 19.3 ± 7.7 (s) in controls) was used to infer reduced muscle mitochondrial function in CF [49]. But, it was highlighted that the use of the pH dependent kinetic parameter,

may have led to an underestimation in the extent of muscle decline in the CF group relative to controls [50]. The standardised, ischemic protocol used in this chapter avoids such issues, albeit at the expense of low end-exercise pH.

### 7.6 References


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# Chapter 8. Discussion

### 8.0 Aims

The purpose of this final chapter is to summarise the novel data generated within this thesis. The first section of this discussion will re-cap the main findings from the experimental chapters, prior to summarising the collective findings. The subsequent sections will focus on the future directions for IBD fatigue research in the context of the data produced within this thesis.

### 8.1 Rationale

The literature review contained within chapter 2 highlighted the current knowledge base pertaining to IBD fatigue aetiology, albeit largely from indirect physiological data given the absence of mechanistic investigation of IBD fatigue aetiology. The main findings highlighted a key role for primary factors related to disease activity in IBD fatigue. This mainly pertains to inflammation [1] and the associated pharmacological treatments targeting inflammatory burden. Although it remains unclear from available data why the increase in fatigue perception and premature exercise fatigue commonly persists in quiescent IBD. Numerous secondary factors were identified that associate with various fatigue domains (i.e. fatigue perception and exercise fatigue), such as malnutrition [2], anaemia [3], sleep disturbances [4] and psychological comorbidities [5]. Although there are no causal data evidencing a direct link between these factors and the development of IBD fatigue. A small number of studies have stratified IBD patients into fatigued and non-fatigued sub groups based upon their fatigue perception scores. These data revealed significant alterations in immune profile [6], faecal microbiota composition [7] and physical deconditioning (i.e. a reduction in $VO_2$ peak and peripheral muscle strength) [8]. This suggests these factors may modulate IBD fatigue. In the context of the potential role of deconditioning in IBD fatigue aetiology, the aim of this thesis

was to combine self-reported fatigue assessment with laboratory-based exercise testing and the more recently developed exercise-MRI theme, to comprehensively phenotype central and peripheral physiology in quiescent IBD patients.

## 8.2 Review of the main findings

### 8.2.1 Body composition, muscle strength and fatiguability in quiescent IBD

During chapter 5, we sought to assess self-reported fatigue perception, body composition and peripheral muscle function in quiescent Crohn's disease patients, relative to an age and BMI matched healthy control cohort. Previous data has evidenced reductions in peripheral muscle strength during instantaneous maximum isometric contractions [9, 10], repeated maximal isometric contractions [11, 12] and during sustained [12] and repeated [13] isometric contractions in IBD cohorts to healthy control groups. We highlighted the more variable findings from data on upper limb strength in IBD patients, where strength loss is reported in some cohorts [2] but maintained in others [12], relative to healthy controls.

In agreement with the loss of peripheral strength, we highlighted the prevalence of IBD sarcopenia [14], now a recognised IBD comorbidity. The review further highlighted available molecular data showing dysregulation of anabolic signalling in skeletal muscle biopsy's of IBD patients compared to that of controls [15]. Thus suggesting a potential mechanistic basis behind the prevalence of sarcopenia in IBD [14] and loss of peripheral muscle strength [2, 10, 12, 13].

In contrast to the majority of available data, we evidenced a maintenance of lean body mass in quiescent Crohn's disease patients relative to healthy controls. Consistently, isometric and isokinetic knee extensor strength was maintained. Thus, our quiescent Crohn's disease cohort reported varying magnitudes of fatigue perception and showed no alterations in body

composition or in peripheral muscle function relative to an age and BMI matched healthy control cohort.

### 8.2.2 Whole-body deconditioning in quiescent IBD patients

Based on the maintenance of body composition and peripheral muscle strength in this Crohn's disease cohort, the aim of chapter 6 & 7 was to ascertain whether these patients with mixed levels of fatigue perception demonstrated whole-body deconditioning, relative to the healthy control group. Data from laboratory based incremental exercise testing was combined with the $^{1}H$ MRI assessment of the central cardiorespiratory and brain vascular and metabolic responses to sustained low intensity exercise. This was followed by the assessment of skeletal muscle mitochondrial function via $^{31}P$ MRS in chapter 7.

We observed a maintenance of cardiorespiratory fitness in the quiescent Crohn's disease group compared to healthy controls. Consistently, the central cardiovascular response to sustained low intensity exercise (i.e. cardiac output during supine stepping exercise), together with $VO_2$, was comparable between quiescent Crohn's disease patients and healthy controls. We report a blunted CBF response to exercise in the Crohn's disease patients, in line with previous reports in fatigueable, deconditioned cohorts such as the elderly [16]. However this cannot be interpreted to reflect an alteration in cerebral neuronal activity and metabolic response [17] to exercise in Crohn's disease patients in the presence of maintained and cardiac output, oxygen extraction fraction and $gmCMRO_2$. The reduction of CBF on exercise is an interesting finding and is consistent with the reduced VE and $VCO_2$ found in Crohn's disease patients relative to the healthy controls during supine stepping at 50% $VO_2$ peak. Although these respiratory and CBF data are difficult to explain in the context of comparable $P_{CO2}$ concentrations, the maintenance of peak $VO_2$, cardiac output, and brain oxygen metabolism responses to standardised, relative intensity exercise. This is in contrast to previous data in the elderly, where

the blunted CBF response to exercise is associated with lower $P_{CO2}$ concentrations [16] and accompanied by a reduction in VE, $VCO_2$ and $VO_2$ compared to healthy young controls [18] during relative intensity matched exercise.

An important consideration when interpreting these data is one of sample size and corresponding statistical power. Unlike the experimental data contained within chapter 5 and 7, where attained sample sizes were sufficient in line with the power calculations outlined in the methods section, there was a significant loss of MRI data in chapter 6 (see results section). This included measurements of aortic cardiac index and to a lesser extent, cerebral blood flow and brain oxygen metabolism. All outcome measurements here fell below the generally accepted target sample sizes for neuroimaging outlined in the methods section [19]. It is important to consider the possibility of a type II error within these results. As a result, the data presented within chapter 6 do not provide conclusive evidence of a maintenance cardiorespiratory and neurometabolic function in quiescent Crohn's disease patients, relative to healthy controls. Future experiments with sufficient sample sizes will aid in confirming this.

On the contrary, our findings from $^{31}P$ MRS experiments provide indirect evidence of peripheral muscle decline in quiescent Crohn's disease patients with variable levels of self-reported fatigue perception. Regardless of the kinetic parameter used to infer muscle mitochondrial function, quiescent Crohn's disease patients showed a slowed rate of PCr resynthesis, reflecting an inhibition of the muscle mitochondria to rephosphorylate ADP, following intense contraction. Comparable loss of muscle oxidative capacity is observed across a host of conditions including insulin resistance [20], type 2 diabetes [21], MS [22], COPD [23] and in ageing [24]. Our findings of diminished muscle oxidative capacity are congruent with the measurements of habitual physical activity reported in chapter 5-7. Here, the mean daily step-count of Crohn's disease patients was significantly less than healthy controls over a 7-day measurement period.

Contrary to previous data, these collective findings provide no evidence of central cardiorespiratory deconditioning in quiescent Crohn's disease patients. Instead, these data strongly point to in-activity induced muscle decline in the absence of muscle atrophy and strength loss. This suggests that the alterations at the skeletal muscle level are specific to the mitochondria (i.e. a loss of mitochondrial content), although muscle biopsy sampling would be required to confirm this hypothesis.

### 8.2.3 CNS changes in IBD

To complete the comprehensive phenotyping of IBD fatigue, in chapter 6 we investigated brain morphometry changes in the CD cohort. The results evidenced a maintenance of both grey and white matter concentrations concomitant to less CSF volume in Crohn's disease patients relative to healthy controls. CSF flow is essential for protein clearance and abnormalities in CSF flow [25] and volume [26] are associated with cognitive function in the elderly and fatigue perception in CFS. Our findings are consistent with other unpublished data from this laboratory, which has demonstrated that the loss of CSF also occurs in active Crohn's disease. These collective findings imply that the loss of CSF in IBD is independent of disease activity. However, the clinical significance of these changes is unclear and require further investigation.

### 8.2.4 Summary of experimental data

Collectively through comprehensive phenotyping experiments of whole-body physiology, we have found that quiescent Crohn's disease patients with varying magnitudes of self-reported fatigue perception, display clear evidence of muscle metabolic decline in the absence of strength loss and muscle atrophy.

For the first time, these data directly probe central and peripheral physiology in quiescent IBD and reveal a reduction in muscle mitochondrial function. This is likely the mechanism underlying the commonly reported performance deficits in IBD.

## 8.3 Future directions in IBD fatigue research

### 8.3.1 Exercise training intervention in IBD fatigue

The data generated from this thesis evidence impaired *in-vivo* muscle mitochondrial function in quiescent Crohn's disease patients. This occurred independently of muscle atrophy, strength loss or cardiorespiratory deconditioning. Paired with the reduced habitual physical activity relative to healthy controls reported in chapter 6, these collective findings are indicative of in-activity induced muscle in this patient cohort. These $^{31}$P MRS data corroborates a plethora of existing data evidencing functional decline in IBD cohorts [8, 9, 12, 27, 28]. Fundamentally, this provides an evidence base for the implementation of treatment interventions to augment muscle mitochondrial function in IBD fatigue.

The most pragmatic treatment strategy to trial in fatigued IBD patients based on the loss of *in-vivo* mitochondrial function would be an exercise training intervention targeting mitochondrial biogenesis. This is a fundamental adaptation to traditional aerobic endurance training [29] leading to an increase in mitochondrial density. The subsequent improvements in the capacity for ATP synthesis increases exercise performance at submaximal workloads [30]. These adaptations are known to be regulated by contraction induced activation of the transcriptional coactivator, peroxisome proliferator-activated receptor (PPAR)γ co-activator 1α (PGC-1α) [31], regarded as the master regulator of mitochondrial biogenesis [32, 33] which increases

mitochondrial content [34] and augments exercise capacity [35]. Multiple modes of aerobic exercise training including traditional high volume, moderate intensity aerobic training and low volume, high-intensity exercise training stimulates muscle mitochondrial biogenesis [36-38].

There is a concern over the exacerbation of gastrointestinal symptoms resulting from exercise participation in IBD patients. However, it has been shown that IBD patients can tolerate exercise modalities at varying relative intensities ranging from sustained cycling at 60% $VO_2$ peak [39] to intermittent cycling at 100% of peak power [40], independent of an exacerbation of disease activity. This supports the implementation of exercise training in IBD.

Aerobic exercise training interventions have previously been trialled in IBD albeit not in the context of alleviating fatigue symptoms. A 12-week pilot RCT compared moderate and high-intensity exercise training in 36 Crohn's disease patients (13 HIIT, 12 MICT and 11 control). The moderate intensity intervention consisted of 30 minutes at 35%-watt peak and the HIIT intervention was 10 x 60s bouts at 90% w peak interspersed with 60s recovery at 15%-watt peak, both were performed thrice weekly. There was a small, significant increase in peak $VO_2$ in the HIIT group (27.3 ± 7.7 vs 29.7 ± 8.2 ml/kg/min) only [41].

More recently, eight weeks of concurrent training in a cohort of 17 IBD patients, which includes the simultaneous application of both aerobic and strength training into a combined paradigm [42] demonstrated a reduction in total body fat percentage relative to baseline following the 8 week training intervention in the exercise group only ($P$<0.01). Lean tissue mass also significantly increased in the exercise group only, following the 8-week intervention ($P$<0.001) [43].

Fatigue perception is yet to be assessed as a primary outcome variable during exercise training interventions in IBD patients. A recent RCT of combined impact and resistance exercise training in quiescent Crohn's disease patients demonstrated a reduction in fatigue perception,

assessed as a secondary outcome by the IBD Fatigue scale, at 6 months post intervention ($P$ = 0.005). This was observed alongside significant increases in lumbar spine BMD ( $P < 0.001$), grip strength ( $P < 0.001$), sit and stand test ability ( $P < 0.001$) and isokinetic strength of the upper ($P < 0.001$) and lower limbs ( $P < 0.001$) [44]. A previous study compared the effect of increased physical activity and/or omega-3fatty acid supplementation on fatigue in a cohort of 52 quiescent IBD patients [45]. The inconclusive results are highlighted in chapter 2, however the lack of objective end-points together with the fact that the exercise intervention consisted solely of an exercise advice consultation, means comparing these data are inappropriate.

Regardless, the improvement in fatigue perception following resistance exercise training is an interesting finding. Although prescribed to target muscle weakness and osteopenia, both of which are common in IBD, the quiescent Crohn's disease patients included in this thesis showed comparable peripheral muscle strength and BMD to healthy controls. The potential reasons for this were discussed in chapter 5. However, it is important to consider these data from a small cohort of patients in the context of the available literature base, which consistently evidences a loss of peripheral muscle strength in IBD. Thus, whilst muscle strength was maintained in our cohort, the importance of resistance exercise training in IBD as a whole, should not be ignored. Primarily, our data suggest that the implementation of aerobic exercise training intervention could target muscle decline and improve fatigue burden in IBD. However, given the potential for selection bias (discussed in chapter 5) in this exercise-based study and the known alterations in peripheral muscle function, and cardiorespiratory fitness in IBD which are related in pathology [46], concurrent training interventions may represent the most pragmatic and theoretically sound management strategy to trial in IBD fatigue.

Exercise training interventions in chronic diseases with a high prevalence of fatigue perception and deconditioning are known to improve fatigue burden and exercise capacity [47, 48]. The aforementioned exercise interventions carried out in IBD patients with a predominant aerobic

component are of a high quality design and consistent with programs shown to stimulate mitochondrial biogenesis [38]. This is congruent with the positive metabolic [43] and functional outcomes [41] reported following from these trials, which are implicit of increases in muscle mass and mitochondrial content. Given these interventions are clearly feasible in IBD, future work should aim to assess the impact of these interventions, ensuring that fatigue perception and muscle mitochondrial function are assessed pre-post as primary outcome variables. Existing studies have assessed cardiorespiratory fitness and peripheral muscle function pre-post training intervention. However, the absence of fatigue measurement together with *in-vivo* assessment of muscle mitochondrial function means that the mechanistic basis of any observed functional improvements and the effect on perceived fatigue burden have not been characterised.

### 8.3.2 The potential role of $^{31}$P MRS in IBD exercise training

$^{31}$P MRS can identify improvements in muscle metabolic quality following exercise training intervention. In 10 MS patients, 8 weeks of high-intensity exercise training evoked a significant increase in $VO_2$ max (30 ± 9.3 vs 33.8 ± 8.5 mL/kg/min, $P < 0.001$), reduced fatigue perception scores on the MFIS scale ( 31.3 ± 188 vs 23.6 ± 17.3, $P = 0.05$) and increased the $k_{PCr}$ in the TA (0.022 ± 0.05 vs 0.028 ± 0.01$s^{-1}$ , $P = 0.03$) relative to a control cohort of 7 patients [49]. Earlier $^{31}$P MRS data also in MS, demonstrated increased fatigue resistance and a decrease in the Pi/PCr ratio at peak exercise workload, together with increased cytosolic pH values following 8 weeks of wrist flexor exercise training [50]. Thus, the application of the $^{31}$P MRS protocol developed within this thesis could be used to quantify training induced improvements in muscle metabolic quality in IBD.

### 8.3.3 Walking interventions

A simple increase in habitual physical activity may also represent a pragmatic approach to target deconditioning in IBD fatigue. A walking intervention was shown to improve glucose tolerance ($P < 0.001$) and blood pressure ($P < 0.001$) independent of alterations in body composition in a cohort of 18 overweight, sedentary woman who increased their daily pedometer measured step count from 4491 ± 2269 to 9213 ± 362 over an 8-week period [51]. However, the sole elevation in habitual physical activity may not be a potent enough stimulus to augment *in-vivo* muscle mitochondrial function. Type 2 diabetics who increased their step-count from 6450 ± 851 to 12322 ± 1979 during an eight week walking intervention showed an improvement in lipid oxidation but no change in the PCr time constant ($t_{PCr}$) from pre-post intervention [52], indicating no change in mitochondrial function. In contrast, 12-weeks of concurrent exercise training in type 2 diabetics including bi-weekly cycling exercise at 55% of pre-determined watt max and a single session of whole-body resistance exercise training at 55-75% of MVC, significantly improved insulin sensitivity ( $P < 0.01$) and *in-vivo* mitochondrial function assessed as the rate constant of PCr recovery following exercise ($k_{PCr}$) ($P < 0.05$) [21].

Walking interventions have been trialled in IBD previously and appear to be well tolerated. A supervised 12 week walking intervention performed thrice weekly in a cohort of 16 quiescent or mildly active CD patients reduced self-reported stress levels ($P = 0.0005$) and disease activity ($P = 0.02$) and increased quality of life ($P = 0.01$) and estimated $VO_2$ peak ($P = 0.0013$) [53]. These data are consistent with a later study involving a low intensity walking intervention, where 32 mildly active or quiescent CD patients were randomised either to the intervention or a control group. The intervention was shown to improve self-reported stress, quality of life ($P < 0.05$) and disease activity ($P < 0.01$) [54]. Reapplication of similar interventions with the addition of objective primary outcome measures pertaining to muscle metabolic health and the

association with fatigue perception would help to elucidate whether this pragmatic strategy can improve muscle decline and self-reported fatigue symptoms in IBD.

### 8.4 Pharmacological approaches to target mitochondrial function in IBD

There is a strong interest in modulating muscle mitochondrial function by pharmacological treatment targets given the prevalence of mitochondrial dysfunction across chronic disease [55]. The basic rationale is that drug-induced stimulation of mitochondrial biogenesis would increase cellular capacity for oxidative phosphorylation. The resultant elevation in capacity for ATP synthesis would therefore enhance the functional capacity of skeletal muscle, as is observed with exercise training [36, 37]. This pharmacological approach is of interest in patient cohorts such as those with mitochondrial diseases, or indeed any chronic disease characterised by muscle deconditioning, where the provision of exercise training intervention may be unviable or impractical.

The data within chapter 7 demonstrated a loss of *in-vivo* muscle mitochondrial function in quiescent Crohn's disease patients, highlighting exercise mimetics as a potential treatment target. Both caloric restriction [56] and multiple modes of aerobic exercise training stimulate muscle mitochondrial biogenesis [36, 37]. This adaptation is known to be modulated by the cellular energy sensor, adenosine monophosphate protein kinase (AMPK) [57]. An increase in the AMP/ATP ratio during increased cellular energy demand activates AMPK [58] which is known to modulate SIRT1 activity by elevating cellular $NAD^+$ concentrations [59] and activates the peroxisome proliferator-activated receptor (PPAR)γ co-activator 1α (PGC-1α) [31], regarded as the master regulator of mitochondrial biogenesis [32].

AMPK can be targeted via aminoimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR). Four weeks of AICAR administration (500mg/kg/day) in mice stimulates AMPK signalling

and increased treadmill running distance by 44% relative to vehicle treated mice, independent of any exercise training [60]. Additionally, targeting the AMPK-SIRT1- PGC-1α signalling complex has demonstrated efficacy in rodent model. In mice that were fed a high fat diet, concomitant resveratrol administration increased citrate synthase activity,$VO_2$ and running distance ( all $P < 0.05$) relative to mice that were only fed a high fat diet [61]. These effects were attributed to an increase in PGC-1α activation. In a human model, 30 days of resveratrol supplementation (150mg/day) in 11 obese males was associated with a decrease in lipid content and an improvement in insulin sensitivity. Resveratrol supplementation stimulated an increase in PGC-1α and SIRT1 protein content and increased citrate synthase activity ($P < 0.05$), but had no effect on in-vivo mitochondrial function, as the PCr time constant in the vastus lateralis was consistent with placebo. Together with the maintenance of mtDNA copy number and OXPHOS protein content, it was postulated that the positive effects of resveratrol treatment were attributable to improvements in mitochondrial efficiency as appose to increased density. Although it was suggested that chronic supplementation may increase mitochondrial content.

### 8.5 Future assessment of CNS changes in IBD fatigue

Combining functional neuroimaging and electrophysiological methods during motor tasks has been utilised in chronic diseases such as MS. For example, relative to healthy controls, fatigued MS patients demonstrate a decline in voluntary activation and impaired cortical activation in motor regions during a sustained MVC [62]. This approach could help to probe neural fatigue mechanisms in IBD. Further, the combination of voxel-based morphometry (VBM) and resting state fMRI methods has also proved fruitful in elucidating the neural alterations modulating fatigue perception in MS [63]. Future application in IBD will aid in probing fatigue aetiology.

### 8.6 Vascular oxygen supply and $^{31}$P MRS

Our experimental $^{31}$P MRS protocol did not consider the role of vascular oxygen supply on mitochondrial function. Limb perfusion is critical to mitochondrial respiration and thus PCr recovery from exercise [64, 65]. It is important to consider potential alterations in muscle capillary density [66] and microvascular function [67] and their role in PCr recovery kinetics. Resting skeletal muscle perfusion negatively correlates with the time constant of PCr resynthesis ( $r$ = -0.41, $P$ < 0.001) [68]. Future assessment of muscle perfusion in IBD studies could be quantified by Near infrared spectroscopy (NIRS) which can be concurrently utilised alongside $^{31}$P MRS measurements to quantify oxyhaemoglobin signal [69]. Thus, informing on $O_2$ supply and utilisation across exercise tasks. Muscle perfusion can also be quantified by intravoxel incoherent motion imaging (IVIM) [70] and diffusion weighted imaging (DWI) [68]. Concurrent application of these additional measurements as proxies of muscle perfusion together with localised $^{31}$P MRS measurements will aid in elucidating whether the loss in oxidative capacity in Crohn's disease patients is solely attributable to a loss of mitochondrial density and function or impairments in muscle perfusion and thus substrate supply.

### 8.7 Concluding remarks

The use of multiple non-invasive metabolic imaging methods during this thesis has enabled comprehensive assessment of whole-body physiology in quiescent Crohn's disease patients. These novel data show a slower rate of PCr resynthesis following standardised, high-intensity, ischemic contraction in CD patients, relative to age and BMI matched healthy volunteers. This occurs despite the maintenance of whole-body and appendicular lean mass, peripheral muscle strength and cardiorespiratory fitness.

Collectively, these data implicate peripheral muscle deconditioning independent of atrophy, peripheral muscle weakness and cardiorespiratory deconditioning in IBD fatigue aetiology. For

the first time, these findings provide a mechanistic basis for the widely reported premature exercise fatigue in IBD. Exercise training interventions or pharmacological exercise mimetics represent a pragmatic treatment target to be trialled in IBD fatigue. Concurrent exercise training represent an attractive intervention candidate which could strike a balance of targeting classical factors relating to IBD aetiology, such as osteopenia [71], sarcopenia [72], disease course [73], clinical outcomes [74] and the muscle metabolic decline reported in this thesis. Recent exercise training interventions in IBD cohorts have solely focused on resistance exercise training modalities in an attempt to target osteoporosis [44], whilst measuring fatigue perception as a secondary outcome. The data in this thesis showed no evidence of peripheral muscle weakness or loss of BMD in IBD. This suggests the sole provision of strength training interventions, based on their efficacy in other chronic disease may be an inefficient strategy. As considered in chapter 2, this highlights the merit of characterising IBD physiology prior to prescribing exercise interventions. Based on the data in this thesis, future IBD exercise studies should quantify peripheral muscle function, *in-vivo* mitochondrial function and fatigue perception as primary outcome variables.

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