UNIVERSITY OF BIRMINGHAM

THE ASSESSMENT OF PHYSICAL FRAILTY AND PHYSICAL ACTIVITY IN END-STAGE LIVER DISEASE

by

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Abstract

Physical frailty in end-stage liver disease (ESLD) is prevalent across North America and has a negative impact on clinical outcomes, yet little is known about the prevalence of physical frailty in ESLD within the United Kingdom (UK). Physical activity and exercise-based interventions seem a plausible option to improve physical frailty, yet there is limited understanding of current habitual physical activity levels in ESLD. Consequently, research studies to date have based interventions around well recognised National guidelines designed for healthy individuals or those with other chronic diseases. Effectiveness of, and adherence to, these interventions have been varied, limiting the translation of research findings into clinical practice.

Through a prospective UK-based observational cohort study, I identified high prevalence (80%) of, and the clinical characteristics (i.e. age and hyponatraemia) that predict, physical frailty in ESLD. Furthermore, the quick and simple to use outcome measures, Liver Frailty Index (LFI) and Duke Activity Status Index, were validated for overall and waiting list mortality in patients assessed for liver transplantation (LT).

Understanding the volume and intensity distribution of physical activity in those with ESLD will help guide future study interventions. As part of our wider observational case-control sarcopenia study, I highlighted the negative discrepancy between volume, and distribution of activity intensity, by using remotely-monitored wrist-worn accelerometery of patients with well-characterised ESLD compared to age/sex matched healthy controls. In particular, those with ESLD did not sustain activity at a moderate intensity for longer than one minute indicating that current exercise advice for those with ESLD (5-10min bouts of moderate

intensity physical activity) may be too ambitious. To enable targeted exercise therapy to those most in need, I investigated the clinical predictors of low physical activity levels. Older age and the presence of refractory ascites were independent predictors of low physical activity, with the LFI being the most robust and clinically useful physical frailty measure to predict low physical activity. Intensity, rather than volume of physical activity was associated with lower physical frailty levels indicating a message of “when you move, move with intensity” may be most beneficial to patients with ESLD. Further research studies should focus on delivering short bursts of higher intensity activity within their exercise interventions to evaluate impact of physical activity on reducing physical frailty in ESLD.

Dedication

This thesis is dedicated to my beautiful daughters Francesca and Ottilie. Thank you for your patience, for keeping me grounded and for making me smile when I needed it most.

'Reach for the stars and your feet on the ground'

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Publications:

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2. **Williams FR**, Lord JM, Berzigotti A, Lai JC, Armstrong MJ. Review article: Impact of Exercise on Physical Frailty before and after Liver Transplantation. *Aliment Pharmacol Ther* 2019, 50(9); 988-1000.

3. **Williams FR,** Vallance A, Faulkner T, Towey J, Kyte D, Durman S, Johnson J, El-sharkawy A, Holt A, Perera MT, Ferguson J, Lord JM, Armstrong MJ. Home-Based Exercise in Patients Awaiting Liver Transplantation: A Feasibility Study. *Liver Transplantation* 2019, 25 (7); 995-1006.

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1. **Williams FR, et al.** Physical Frailty predicts liver transplant waiting list mortality: a UK perspective. **British Transplantation Society Annual Conference**; March 2020. Belfast, Northern Ireland.

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LIST OF ABBREVIATIONS

A

AASLD – American Association for the Study of Liver Disease

ACSA – anatomical cross-sectional area

ACSM – American College of Sports Medicine

ADLs – Activities of daily living

AF – Atrial fibrillation

AHPs – Allied Health Professionals

AID – Autoimmune liver disease

AIH – Autoimmune hepatitis

ALP Alkaline phosphatase

αKG – Alpha-ketoglutarate

ALT – Alanine transaminase

AMA – Antimitochondrial antibodies

ANA – Antinuclear antibody

ArLD – Alcohol-related liver disease

AT – Anaerobic threshold

ATP – Adenosine triphosphate

AUROC – Area under the receiver operating characteristic

AWGS – Asian Working Group for Sarcopenia

B

B – Beta coefficient

BCAA – Branch chain amino acids

BRC – Biomedical Research Centre

C

CCGs – Clinical Commissioning Group

CI – Confidence interval

CKD – Chronic kidney disease

CLD – Chronic liver disease

CLDQ – Chronic liver disease questionnaire

CM – Centimetre

CM – Continuous minutes

COPD – Chronic Obstructive Pulmonary Disorder

COVID-19 – Coronavirus-19

CPET – Cardiopulmonary exercise testing

CPS – Child-Turcotte-Pugh Score

CRF – Clinical Research Facility

CS – Chair stands

CT – Computed tomography

CVD – Cardiovascular disease

D

DASI – Duke activity status index

DEXA – Dual energy x-ray absorption

DM – Diabetes Mellitus

DNA – Deoxyribonucleic acid

E

EASL – European Society for the Study of Liver Disease

EFOV – Extended field of view

EME – Efficacy and mechanism evaluation

ENMO – Euclidean norm minus one

ERAS – Enhanced recovery after surgery

ESCID – Evaluation of sarcopenia in chronic inflammatory disease

ESLD – End-stage liver disease

EWGSOP – European Working Group for Sarcopenia

F

FBC – Full blood count

FEV1 – Forced expiratory volume in one minute

FITT – Frequency, intensity, time, type

FraiLT – Functional Assessment in Liver Transplant

Foxo O – Forkhead box O

FVC – Forced vital capacity

G

g/dL – Grams per decilitre

GGT – Gamma-glutamyl transpeptidase

H

HBEP – Home-based exercise programme

HC – Healthy control

HCC – Hepatocellular carcinoma

HGS – Hand grip strength

HIIT – High intensity interval training

HR – Hazard ratio

HTN – Hypertension

I

IA – Inflammatory arthritis

IADLS – Instrumental activities of daily living

IBD – Inflammatory bowel disease

ICU – Intensive care unit

IGF-1 – Insulin-like growth factor

IHD – Ischaemic heart disease

IRF – Inflammation Research Centre

IL-6 – Interleukin-6

INR – International normalised ratio

IPAQ – International physical activity questionnaire

IQR – Interquartile range

IQS – Isometric quadriceps strength

ISWT – Incremental shuttle walk test

K

KPS – Karnofsky performance status scale

Kg – Kilogram

L

LFI – Liver frailty index

LT – Liver transplant

M

M – Metres

MAMC – Mid-arm muscle circumference

MCS – Mental component score

MD – Mean difference

MELD – Model for end-stage liver disease

MELD-Na – Model for end-stage liver disease – sodium

METs – Metabolic equivalents

Mg/dL – Milligrams per decilitre

M*g* – Milli-gravitational units

MIPA – Moderate intensity physical activity

ml – Millilitres

MRI – Magnetic resonance imaging

mTOR – Mammalian target of rapamycin

m/sec – metres per second

MVPA – Moderate to vigorous physical activity

N

N – Number

NAFL – Non-alcohol fatty liver

NAFLD – Non-alcohol fatty liver disease

NASH – Non-alcohol steatohepatitis

NF_kB – Transcription factor nuclear $_kB$

NHS – National Health Service

NHSBT – National Health Service Blood and Transplant

NIHR – National Institute of Health Research

NLR – Neutrophil to lymphocyte ratio

Nm – Newton metre

NT Pro-BNP – N-terminal pro B-type natriuretic peptide

O

OR – Odds ratio

P

PBC – Primary biliary cholangitis

PCS – Physical component score

PICS – Prescribing information communication system

PIS – Participant information sheet

PKB/AKT – Protein kinase B

PPIE – Patient and Public Involvement and Engagement

PSC – Primary sclerosing cholangitis

Q

QEUHB – Queen Elizabeth University Hospitals Birmingham

Quads - Quadriceps

R

RCT – Randomised control trial

REC – Research ethics committee

RIR – Repetitions in reserve

ROS – Reactive oxidative species

RPE – Rate of perceived exertion

S

SD – Standard deviation

SEM – Standard error of the mean

SF-36 – Short-form 36

SPPB – Short physical performance battery

T

TB - TheraBand

TCA – Tricarboxylic acid

TGF-β – Transcription growth factor beta

TNF-α – Tumour necrosis factor alpha

TSF – Triceps skin fold

U

Ub – Ubiquitin

UK – United Kingdom

UKELD – United Kingdom model for end-stage liver disease

US – Ultrasonographic

US – United States

USA – United States of America

V

VIF – Variance inflation factor

VIPA – Vigorous intensity physical activity

VL – Vastus lateralis

VO_2peak – Peak oxygen consumption

W

W – Watts

WBC – White blood cell count

WHO – World Health Organisation

NUMERICAL

6MWD – Six-minute walk distance

6MWT – Six minute walk test

CHAPTER 1:
GENERAL INTRODUCTION

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Williams FR, Milliken D, Lai JC, Armstrong, MJ. Review Article: Assessment of the Frail Patient with End-stage Liver Disease: Overview of sarcopenia, physical function and disability. *Hepatology communications* 2021, 5(6); 923-937.

and

Williams FR, Lord JM, Berzigotti A, Lai JC, Armstrong MJ. Review article: Impact of Exercise on Physical Frailty before and after Liver Transplantation. *Aliment Pharmacol Ther* 2019, 50(9);988-1000.

1.1 Chronic liver disease

Chronic liver disease (CLD) is a global healthcare challenge which accounts for approximately 2 million deaths per year worldwide (1). CLD has a high economic burden, is associated with high hospital admissions and unemployment (2), and accounts for approximately 62,000 working years lost within the United Kingdom (UK) alone (3). In comparison to other major diseases, where disease-related death has been on the decline (i.e. cardiac), CLD-related deaths have risen by 400% over the last five decades within the UK, demonstrating the urgent need for improved liver disease care (3). Indeed, CLD is the second commonest cause of death in males of working age in the UK.

CLD is a term given to a group of heterogeneous diseases characterised by reduced hepatic impairment as a result of chronic injury (i.e. viral, toxins, autoimmunity, metabolic) to the liver (4). In the early stages of CLD, reversible inflammation and subsequent fibrosis (i.e. accumulation of extracellular matrix proteins and fibroblast infiltration) occur as a result of the wound healing response to a liver injury (4-6). If left untreated, the perpetual activation of inflammation and wound healing causes structural development of regenerative nodules that are encompassed by fibrous bands; also known as cirrhosis (7, 8). This alteration in liver architecture and eventual distortion of hepatic vasculature leads to hepatic dysfunction and portal hypertension, termed end-stage liver disease (ESLD). ESLD is made up of two stages; compensated and decompensated cirrhosis (i.e. liver failure). Those with compensated cirrhosis remain largely asymptomatic but may present with non-specific symptoms such as right upper quadrant pain, fatigue, tiredness and pruritus (9). Decompensated cirrhosis is the term used to define progression to hepatic dysfunction (jaundice, coagulopathy, hepatic

encephalopathy) and/or complications of portal hypertension (ascites, spontaneous bacterial peritonitis, variceal haemorrhage) (10, 11). This is an irreversible stage of CLD, with liver transplantation (LT) currently being the only curative option (12) (**Figure 1.1**).

Shortfalls in liver organ donation have resulted in LT waiting list mortality remaining relatively high (7%) (13). To minimise LT waiting list mortality, and ensure prioritisation of available organs, prognostic biomarkers of disease severity and mortality, such as the Child-Turcotte-Pugh score (CPS), Model for End-Stage Liver Disease (MELD), and United Kingdom Model for End-Stage Liver Disease (UKELD) have been developed (14-16). The CPS categorises patients into three grades: A, B and C, with C describing the most severe cases of liver disease (**Table 1.1**). It was originally used to predict outcome in portacaval shunt surgery and later to predict progression of cirrhosis (17, 18). However, the CPS came under criticism due to the subjective assessment of ascites and hepatic encephalopathy, and the lack of accountability for renal function, a well-established prognostic marker in ESLD (19). As such, the MELD, consisting of dialysis needs, international normalised ratio (INR), serum creatinine/dialysis requirement and bilirubin was developed, which has been shown to accurately predict mortality in cirrhosis (20). Yet, it has been highlighted that the inclusion of renal function may be too highly weighted by creatinine in the MELD, which can be influenced by many extrarenal factors, such as gender, ethnicity and muscle mass (21). Furthermore, it does not account for complications of cirrhosis such as hepatic encephalopathy, refractory ascites and variceal bleeding. In 2002, serum sodium was added to the MELD (MELD-Na), due to the association between serum sodium with hepatorenal syndrome, ascites and death in decompensated cirrhosis (22, 23). MELD-Na has since been shown to be a better statistical predictor for risk

than the standard MELD, still it has only been validated retrospectively, whereby the obtainment of serum sodium may have been subjected to laboratory variation (24).

Table 1.1 Child-Turcotte-Pugh Score Classification

Clinical parameters	**Points**		
	1	2	3
Albumin (g/dL)	>3.5	2.8-3.5	<2.8
Bilirubin (mg/dL)	<2	2-3	>3
Prothrombin times (secs prolonged)	<4	4-6	>6
OR			
International normalised ratio	<1.7	1.7-2.3	>2.3
Hepatic encephalopathy*	None	Grade 1-2	Grade 3-4
Ascites	None	Mild-Mod^	Severe^^

*Grade based upon West Haven scale (Villstrup et al. 2014)
^Diuretic controlled
^^Diuretic refractory
Note:
Child-Turcotte-Pugh score obtained by adding the score for each parameter (total points)
Class A = 5-6 points
Class B = 7-9 points
Class C = 10-15 points

More recently, in 2008, the UKELD (INR, serum creatinine, bilirubin, sodium) was developed. A score of ≥49 predicts a 9% one year mortality without a LT, and is the minimum score for listing a patient with cirrhosis for a LT within the UK, who has cirrhosis (16). Whilst all of these scores have been successful in prioritising patients for LT thus far, they concentrate mainly on mortality outcomes. More frequently clinicians are receiving feedback that "living well" is more important than surviving. Specifically, in a National Institute for Health Research (NIHR) Efficacy and Mechanism Evaluation funded study, of which I am site Principal Investigator for, patients within our Patient and Public Involvement and Engagement (PPIE) groups across two LT sites unanimously reported that quality of life while waiting for, and after, a LT was the most valued outcome. Therefore, quality of life and physical frailty may be an important component of organ allocation in the future, yet research in this area is currently scarce. In this regard, a frailty scoring system has been developed for patients with CLD, the Liver Frailty

Index (LFI, see also section 1.2). A study by Lai and colleagues found that for every one point increase in the LFI, there was a 2.6 higher odds of having difficulty with at least one activity of daily living (ADLs) (25). In view of these findings, and the ability of the LFI to predict waiting list mortality, Kardashian et al. investigated the addition of a frailty marker to MELD-Na to predict LT waiting list prognosis. The investigators found that LFI+MELD-Na more accurately represented waiting list mortality than MELD-Na alone (area under the curve (AUC) 0.79 versus 0.73, respectively) (26). These findings indicate the need for clinicians to think more broadly, particularly in terms of frailty, when designing future prognostic models for patients listed for LT.

1.1.1 Aetiology of CLD

The aetiology of CLD can stem from environmental, societal and lifestyle factors such as; alcohol-related liver disease (ArLD) and non-alcoholic fatty liver disease (NAFLD), or non-lifestyle orientated diseases such as: viral, autoimmune and genetic liver diseases (27). Within this thesis participants were recruited with predominantly ArLD, NAFLD and autoimmune liver diseases (AID), and therefore these disease processes have been discussed in more detail below.

1.1.1.1 Alcohol-related Liver Disease

ArLD occurs as a result of an ethanol-mediated liver injury (28). Ethanol is absorbed through the gastrointestinal tract and metabolised in the hepatocytes in the liver, leading to elevated levels of the highly toxic chemical acetaldehyde (29). Acetaldehyde increases DNA synthesis impairment, oxidative stress, inflammation, fatty acid accumulation (i.e. steatosis), and directly damages mitochondria and microtubules within hepatocytes, resulting in steatosis

(30). Chronic ethanol consumption exacerbates these molecular cycles leading to chronic inflammation (i.e. hepatitis), fibrosis and eventually cirrhosis (31). ArLD is suspected in the presence of the combination of regular alcohol consumption (>30g/day (men), 20g/day (women)) and evidence of clinical and/or biological indication of liver injury (32). However, a liver biopsy is the 'gold' standard measure to determine the exact stage and prognosis of ArLD and to rule out any other coexisting liver disease aetiologies (i.e. haemochromatosis, viral hepatitis B/C) (32). Progression of ArLD is usually related to the amount and duration of alcohol use. However, other factors such as genetics, epigenetics and environmental factors (i.e. obesity) can influence disease advancement (30).

1.1.1.2 Non-alcohol related Fatty Liver Disease

NAFLD is the most common liver disease in the Western world, affecting approximately 25% of the population, resulting in high socio-economic burden (33). NAFLD can be divided into two subclassifications; (1) Non-alcoholic fatty liver (NAFL), i.e. the presence of steatosis with mild lobular inflammation or (2) Non-alcoholic steatohepatitis (NASH), i.e. significant steatosis-related inflammation, apoptosis and progressive fibrosis. NASH can be divided into four stages: none or mild fibrosis (Kleiner stage F0-1); significant fibrosis (F2); advanced bridging fibrosis (F3) and NASH cirrhosis (F4) (34). NAFLD is highly associated with insulin resistance and metabolic syndrome and should therefore be considered in anyone with three of the five metabolic syndrome features (i.e. hypertension, central obesity, impaired fasting glucose, type II diabetes myelitis, or low high-density lipoprotein) (34, 35). The diagnosis of NAFLD is generally based upon evidence of steatosis on imaging and/or incidental transaminitis in the absence of excess alcohol consumption (<14/21 units/week in

females/males respectively) and any other cause of liver disease (hepatotoxic drugs and blood serology for viral, autoimmune and inherited diseases) (34, 35).

1.1.1.3 Autoimmune Liver Disease

Autoimmune liver disease is a chronic hepatobiliary disorder with three main clinical presentations; Primary Biliary Cholangitis (PBC), Primary Sclerosing Cholangitis (PSC), and Autoimmune Hepatitis (AIH) that may present in isolation or rarely overlap with one another (36). Exact causes of AID are currently unknown; however, evidence that environmental factors such as, bacteria, viruses and xenobiotics, and genetic predisposition has been found (37). The two main diseases included in this thesis are PBC and PSC and are discussed further below.

PBC is an adult only, female-predominant cholestatic liver disease (9:1) that usually presents in people older than 40 years (37). It is a progressive disease with the development of biliary fibrosis and, if left untreated or resistant to treatment (i.e. ursodeoxycholic acid), leads to biliary cirrhosis and its associated complications (38, 39). PBC is characterised by a sustained elevation (> 6months) above the upper limit of normal for serum alkaline phosphatase (ALP) and serologic reactivity to antimitochondrial antibodies (AMA). If a liver biopsy is completed, a chronic non-suppurative, granulomatous, lymphocytic small bile duct cholangitis will be seen, although a liver biopsy is not always needed (38, 39).

PSC represents the clinical presentation of inflammation, fibrosis and destruction of the intra- and extra hepatic bile ducts which results in cholestasis, bile duct strictures and hepatic fibrosis in the absence of secondary sclerosing cholangitis causes (i.e. chronic obstructive,

immune mediated, infectious, ischaemic, hereditary or toxic) (40, 41). PSC is often associated with Inflammatory Bowel Disease (IBD) which is clinically seen in 50-80% of patients with PSC (42). Whilst PSC is a relatively rare condition (incidence = 1 per 100,000 per year [Europe]) (40), there is an inherent risk of cholangiocarcinoma and colorectal cancer (43-45). PSC is more prevalent in males and tends to present in the 4th-5th decade of life (46). Whilst patients with PSC can live for many years, uncertainties around timing of disease progression, fatigue, pruritus, sleep deprivation and anxiety around risk of cancer mean that poor quality of life is prevalent in patients with PSC (47).

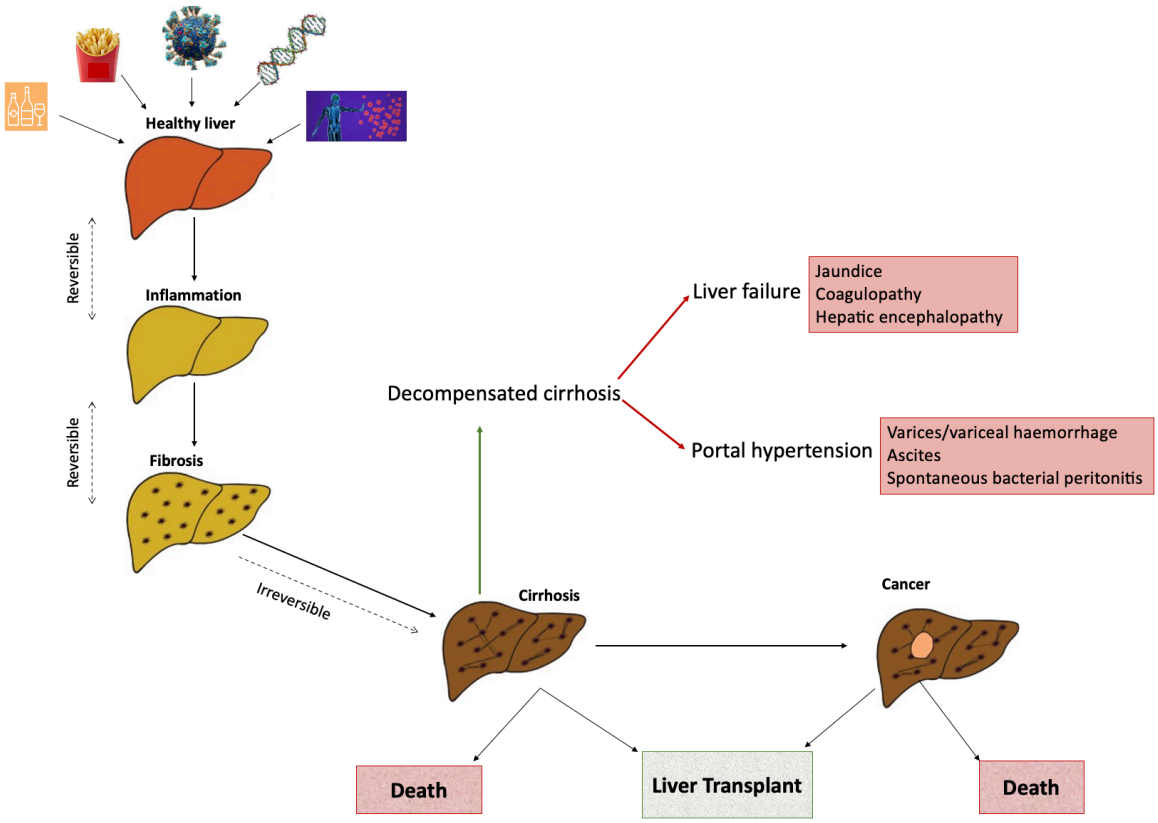


Figure 1.1 Progression of chronic liver disease
An illustration of the causes of chronic liver disease (alcohol, diet, viral, genetic, autoimmune) and how, through repetitive injury and activation of the wound healing process, inflammation can progress to fibrosis and eventually cirrhosis. The clinical characteristics of decompensated cirrhosis are also displayed.

1.2 Frailty in chronic liver disease

Frailty is most commonly defined as a clinical state of decreased physiologic reserve and increased vulnerability to health stressors, which in turn predisposes individuals to adverse clinical outcomes (48). Frailty was first described in community-dwelling adults over the age of 65, as a multi-dimensional construct consisting of physical, psychological, social and other environmental components (48).

Over the last decade, frailty has emerged as a powerful predictor of clinical outcomes in patients with cirrhosis and in those requiring a LT (49-51). Frailty has become more relevant over time as patients with cirrhosis are older in age, sicker as assessed by liver disease severity, and are burdened by co-morbidities including obesity and type 2 diabetes (52). Increasingly, clinicians have recognised the end manifestation of all of these factors in the patient as "frailty" and incorporated an assessment of frailty using the 'eyeball test' into their clinical decision-making (e.g. candidacy for critical care or transplantation). Even though this subjective clinical assessment of frailty has been shown to predict mortality reasonably well in patients with ESLD (53), it lacks objectivity, consistency, reproducibility, and meaningful serial variability. Consequently, recent years have seen the emergence of objective measures of frailty, in particular physical frailty, to assist the high-stake decision-making with ESLD.

1.2.1 Physical frailty

Despite physical frailty being the most frequently described component of frailty in ESLD, there remains a lack of consensus regarding the definition in this patient population. In age-related frailty there are well validated indices such as the Fried Frailty phenotype which has five elements (unintentional weight loss, slow walking speed, weakness or low hand grip

strength, self-reported exhaustion and low physical activity), a score of one or two is deemed a pre-frail state with three or more indicating frailty. In general, in ESLD, physical frailty is not synonymous with, but encompasses; (1) sarcopenia, (2) reduced physical function, (3) reduced aerobic capacity, and (4) reduced functional independence.

1.2.1.1 Sarcopenia

Sarcopenia was first described in 1989 to outline the progressive and generalised loss of skeletal muscle mass in the ageing population (primary sarcopenia) and is now widely recognised in a variety of chronic diseases (secondary sarcopenia), including ESLD (54). However, discrepancies in the definition of sarcopenia exist worldwide. There is a mutual consensus from the European Working Group on Sarcopenia of Older People (EWGSOP) and the Asian Working group for Sarcopenia (AWGS) that sarcopenia can only be diagnosed in the presence of both loss of muscle mass and muscle strength, with the degree of loss of physical function determining the severity of sarcopenia (54, 55). However, there is no consensus on the definition of sarcopenia in Europe and Asia for the diagnosis of sarcopenia in ESLD. In contrast, the American Association for the Study of Liver Disease (AASLD) define sarcopenia as the loss of muscle mass alone (56). Subsequently, the only validated definition of sarcopenia in ESLD relies solely on CT-measured skeletal muscle area at the 3rd lumbar vertebrae, which is normalised to the second power of height to form the 'skeletal muscle index' (57).

The lack of universal criteria for sarcopenia makes it difficult to determine the exact world-wide prevalence and severity of sarcopenia in ESLD. Nevertheless, prevalence has been reported between 22% and 70% with those with one or more components of sarcopenia

being at higher risk of poor clinical outcomes such as: mortality (58), hospitalisation (59), infections (60), extended hospital length of stay (59), encephalopathy (61), and reduced quality of life (62).

1.2.1.2 Reduced physical function

Reduced physical function is a progressive decrease in muscle strength (e.g. hand grip strength [HGS]) and/or function (e.g. chair stands). Physical function in patients with ESLD declines over time with low physical function associated with waiting list mortality and/or de-listing, independent of liver disease severity (50).

1.2.1.3 Reduced aerobic exercise capacity

Reduced aerobic capacity is a deficient utilisation of oxygen, leading to a reduced capacity to sustain physical work or endure physiological stresses including major surgery (63). Typically, aerobic exercise capacity is assessed through direct measurement of oxygen consumption by a patient on a treadmill or cycle ergometer, or by indirect measures such as field walking tests. Patients listed for LT have reduced aerobic capacity with those with lower aerobic capacity at higher risk of pre-and post-LT mortality (64).

1.2.1.4 Reduced functional independence

Reduced functional independence describes deficits in the ability to complete activities necessary to live independently within one's home and in one's community, commonly known as ADLs and instrumental ADLs (IADLs), respectively (49). 43% of patients on the LT waiting list can have difficulty with one or more ADL such as shopping, laundry or housekeeping (49). The level of functional difficulty may vary between disease aetiologies and

patients, for example, an older patient with ArLD and ascites is more likely to be functionally impaired than a younger patient with autoimmune disease who has not yet presented with symptoms of end-stage liver disease. Nevertheless, any difficulty with ADL can not only increases patient dependence on other individuals, but can lead to compromise in safety, reduced quality of life and premature mortality (25, 65).

1.3 Regulation of muscle mass in the normal state

The development of physical frailty seen in patients with ESLD can be explained by the multiple mechanistic and clinical causes of muscle dysfunction. To understand these mechanisms, one must first comprehend the regulation of muscle in the normal state. In the healthy state muscle mass remains relatively constant due to the balance between the rate of muscle protein synthesis, muscle protein breakdown and satellite cell differentiation and proliferation (66). An overview of each of these pathways is described below and in Figure 1.2.

1.3.1 Muscle protein synthesis

Muscle protein synthesis is induced by anabolic signals acting through the mammalian target of rapamycin (mTOR) intracellular signalling pathway. Protein kinase B (PKB/AKT) is upregulated by anabolic stimuli including insulin-like growth factor (IGF-1), insulin, testosterone, physical activity and branch chain amino acids (BCAA) (particularly leucine), which activates the mTOR pathway. In addition, repeated muscular contraction activates the mTOR pathway, not via P13k/AKT, but via the release of phosphatidic acid from the activation of phospholipase D (enzyme found within z-bands) and/or zeta isoform of diacylglycerol kinase during muscular contraction (67). In turn, activation of the mTOR pathway leads to

phosphorylation of the translational initiation factors 4EBP and p70S6K stimulate translation and increase muscle protein synthesis (68). Furthermore, the protein ULK1 inhibits autophagy (removal of damaged organelles and proteins), resulting in maximal muscle growth (69) (Figure 1.2).

Muscle protein breakdown is activated in the presence of glucocorticoids, systemic inflammation and impaired insulin/IGF-1 signalling and occurs as a result of inhibition of the PKB/AKT pathway (70). Inhibition of PKB/AKT increases translocation of the transcription factor Forkhead box O (Foxo) from the cytoplasm to the nucleus, which then induces expression of atrophy-related genes in muscle such as Fbxo32 (atrogin1), Trim63 (MuRF1) as well as autophagy genes (i.e. FoxO3) (70). Consequently, three major proteolytic systems are stimulated; 1) adenosine-triphosphate (ATP)-dependent ubiquitin-proteasome, 2) caspase-mediated protein cleavage and 3) autophagy (69) (Figure 1.2).

The ubiquitin-proteasome system involves the activation of ubiquitin-activating enzyme E1 which transfers to a ubiquitin (Ub) conjugating enzyme E2. In turn, E3 ligases (atrogin 1/MuRF-1) attach the E2-Ub to protein substrates for degradation. Casapse-3 recognises E2-Ub bound proteins and breaks down their myofibril structure releasing easily digestible filaments for 26S proteasome to degrade into monomers and eventually amino acids (71).

Autophagy is the process by which double-membrane vesicles, known as autophagosomes, bulk around cytoplasm, organelles (e.g. mitochondria) and proteins and transport them to the lysosome for degradation (72). Autophagy is vital for removing old and damaged cellular components, breaking down undedicated nutrient stores and remodelling cellular

architecture (73). When the activation/inhibition of PKB/AKT is unbalanced in favour of activation of Foxo, autophagy is accelerated and muscle atrophy occurs.

1.3.2 Satellite cell differentiation and proliferation

Satellite cells are a group of myogenic precursor cells located between the basal lamina and the sarcolemma of the muscle fibre (74). Once activated, satellite cells proliferate and differentiate into myoblasts which then fuse with existing myofibers to repair damaged muscle and/or facilitate an increase in muscle size (74). The presence of myostatin causes negative regulation of satellite cells, keeping them in a dormant state, which leads to overall muscle protein breakdown. However, myostatin can be inhibited by the activation of PKB which maintains satellite cell activity (75).

1.4 Causes of physical frailty in end-stage liver disease

1.4.1 Mechanistic causes of physical frailty in ESLD

The mechanism of physical frailty in ESLD is complex and multi-factorial. Mechanistic causes stem from, but are not limited to: chronic inflammation, malnutrition, endocrine dysfunction, and hyperammonaemia (66, 76-78).

1.4.1.1 Chronic inflammation

Myokines such as myostatin, interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-α) are cytokines synthesised in the skeletal muscle tissue in response to muscular contractions (79). Myostatin is a member of the transcription growth factor beta (TGF-β) family which inhibits muscle protein synthesis and satellite cell activity as well as increasing proteolysis by

inhibiting the AKT/mTOR pathway (80). Elevated levels of myostatin in liver cirrhosis result in skeletal muscle loss (80) and have been associated with lower survival (81).

The local production of IL-6 during skeletal muscle contraction promotes muscle growth by increasing proliferation of satellite cells and thereby regeneration of damaged myofibers (82). In pro-inflammatory conditions, such as ESLD, elevated systemic levels of IL-6 are found, largely due to their production outside of muscle, for example by immune cells or adipose tissue. Although the underlying mechanism is not fully understood, it is thought that the sustained elevation of IL-6 in these patients results in a series of biological responses which causes skeletal muscle atrophy (83).

Similarly, the pro-inflammatory state found in ESLD results in a sustained raised level of TNF-α (84). TNF-α triggers the ubiquitin-proteasome pathway by activating transcription factor nuclear $_{K}$B ($NF_{K}B$). $NF_{K}B$ activates MuRF1 and atrogin1 stimulating muscle protein breakdown (85). In addition, although not proven in ESLD it has been shown that pro-inflammatory cytokines induce expression of the enzyme 11-βHSD1 which generates cortisol in tissues including muscle, with profound catabolic effects (86, 87).

1.4.1.2 Malnutrition

Protein malnutrition leads to reduced substrate availability for muscle protein synthesis, thus plays an important role in sarcopenia in CLD. Malnutrition has been reported to be present in up to 50% of patients with decompensated cirrhosis (88) and is caused by numerous factors, such as: reduced protein intake, malabsorption, altered protein/energy metabolism and accelerated starving (76, 78). Reduced oral intake may result from nausea and anorexia,

caused by raised inflammatory mediators such as TNF-α, impaired gastric expansion from ascites, dysgeusia due to zinc deficiency, abdominal pain and altered gut motility (76). Furthermore, patients frequently present with fatigue (i.e. in autoimmune mediated liver disease) and/or an altered conscious state (hepatic encephalopathy) which can lead to patients forgetting, or lacking in energy, to prepare nutritionally balanced meals.

A net negative energy balance in cirrhosis also occurs due to fat malabsorption. This is caused by a reduction of luminal bile acids secondary to decreased synthesis, portosystemic shunting and pancreatic insufficiency, in those with chronic alcohol consumption (89). Malabsorption of nutrients may also be caused by portal hypertension, due to microcirculatory changes in the gastric mucosa (90), intestinal dysbiosis and chronic lactulose use (89).

Altered macronutrient metabolism is one of the biggest contributing factors to malnutrition in cirrhosis (88). Chronic alcohol consumption in particular stimulates lipogenesisCarbohydrate metabolism is impaired in patients with liver cirrhosis due to peripheral insulin resistance, hyperinsulinemia and impaired hepatic glycogen synthesis, resulting in poor hepatic glycogen stores and a state of accelerated starvation (91). Therefore, alternate sources, such as fatty acids, are needed to generate glucose. However, hepatic cellular dysfunction reduces the uptake of glycerol from lipolysis, therefore limiting gluconeogenesis and increasing myosteatosis (92). Subsequently aromatic and BCAA are obtained from skeletal muscle protein breakdown. This process is further exacerbated by an overall increase in resting energy expenditure, in part driven by chronic upregulation of inflammatory mediators (78). Furthermore, recurrent proteolysis results in reduced circulating BCAA and anabolic resistance. This causes insufficient replenishment of protein

stores in the next fed state, impairing muscle protein synthesis further, resulting in skeletal muscle atrophy (93).

1.4.1.3 Endocrine dysfunction

1.4.1.3.1 Insulin Resistance

In skeletal muscle, insulin stimulates muscle growth and protein synthesis via the MAPK and P13K/AKT2 pathways respectively, and inhibits activation of the Foxo pathway, thereby inhibiting muscle protein breakdown (94). Insulin resistance contributes to impairment in glucose metabolism in patients with liver cirrhosis, with 80% having impaired glucose tolerance and approximately 20% developing overt type II diabetes (95). Insulin resistance results in reduced activation of the MAPK and P13K/AKT2 pathway leading to a reduction in muscle protein synthesis and growth.

1.4.1.3.2 Testosterone

Testosterone inhibits myostatin production, which leads to an increase in IGF-1 levels, and activation muscle protein synthesis via the mTOR pathway (96). However, low testosterone levels are prevalent being seen in up to 90% of male patients with liver cirrhosis and are attributed to: (a) defects at all levels of the hypothalamic-pituitary-testicular axis and (b) increased binding of testosterone to sex hormone binding globulin (97). Additionally, in a study conducted by Sinclair and colleagues, low levels of testosterone correlated with low muscle mass and overall mortality in men with cirrhosis (96).

1.4.1.4 Hyperammonemia

Hepatocellular dysfunction and portosystemic shunting impair the removal of ammonia through ureagenesis (76). Hyperammonemia promotes muscle protein breakdown in three pathways: (a) activation of myostatin; (b) increase in oxidative stress and impaired mitochondrial function; (c) increased skeletal muscle autophagy. As previously described, activation of myostatin inhibits muscle protein synthesis, impairing skeletal muscle growth and reducing muscle mass. The increase in oxidative stress and mitochondrial dysfunction results from the mechanism of ammonia disposal by the skeletal muscle. Skeletal muscle ammonia is normally metabolised by glutamate dehydrogenase which catalyses the α-ketoglutarate (αKG) to glutamate within the muscle mitochondria by anaplerotic conversion (98). However, during a state of hyperammonaemia, cataplerosis (loss of tricarboxylic acid (TCA) cycle intermediates by enzymatic reactions) of αKG is favoured, which results in impaired mitochondrial electron transport chain components (i.e. altered NAD+/NADH ratio) and subsequently reduced ATP synthesis (99). Since muscle protein synthesis is an energy intense process, low ATP concentrations can reduce muscle protein synthesis (66). Furthermore, BCAA are needed to generate the glutamate needed for ammonia detoxification. The increased uptake of circulating BCAA by skeletal muscle further reduces the availability of serum BCAA needed for the stimulation of muscle protein synthesis, inhibition of proteolysis and muscle autophagy (76). Therefore, patients who have cirrhosis and sarcopenia are likely to have reduced circulating BCAA and low muscle mass which, in turn, will limit ammonia clearance and lead to complications such as hepatic encephalopathy.

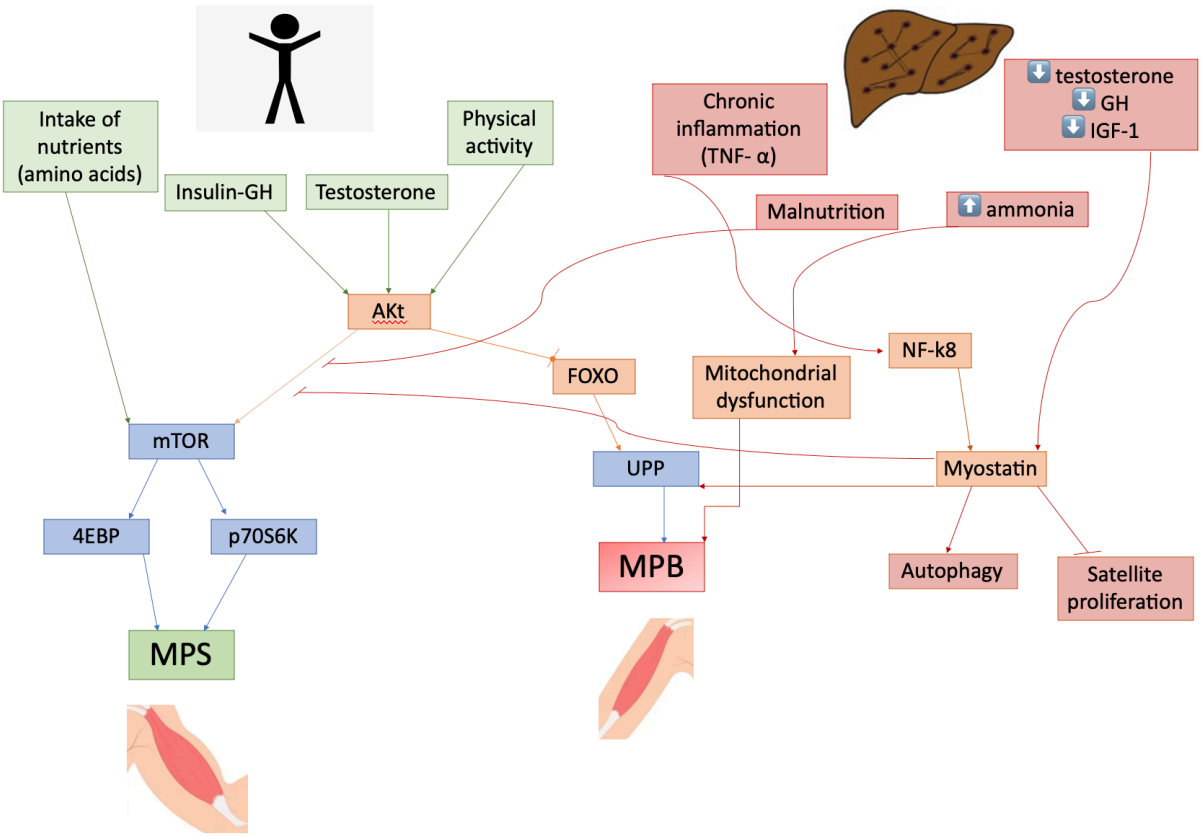


Figure 1.2 Regulation of muscle mass in the healthy adult and in the presence of CLD
Anabolic stimuli such as insulin-like growth factor (IGF-1), testosterone, physical activity and amino acids activate the mTOR intracellular pathway resulting in muscle protein synthesis. In the presence of CLD, mechanisms such as chronic inflammation, malnutrition, hyperammonaemia and endocrine dysfunction increase myostatin production which inhibits muscle protein synthesis and satellite cell activity while triggering the ubiquitin-proteasome pathway and autophagy

1.4.2 Complications of end-stage liver disease which contribute to physical frailty

Once cirrhosis is established, patients are at high risk of developing complications such as hepatic encephalopathy, ascites, and variceal bleeding, moving them into the decompensated stage (11). The former two of which will be discussed below.

1.4.2.1 Hepatic encephalopathy

Hepatic encephalopathy, a state of neurocognitive and psychiatric dysfunction caused by liver insufficiency and/or portosystemic shunting, is a common debilitating complication of liver cirrhosis, which impacts negatively on both patients and their caregivers (100). The West

Haven criteria is most often used to grade hepatic encephalopathy (100), but in its mildest form, subclinical changes such as inattention, anxiety, and sleep disturbances occur (101, 102). As the severity progresses clinical signs of lethargy, marked disorientation and confusion ensue, with the eventual progression to a coma (103). Hepatic encephalopathy can occur spontaneously or following a clinical event such as infection, variceal bleed, constipation and dehydration (104). Between episodes, patients can return to their baseline status. In ESLD, minimal hepatic encephalopathy is prevalent in the majority (up to 80%) of patients with decompensated cirrhosis, yet overt hepatic encephalopathy can still affect a substantial proportion (20-40%) of these patients (100). More recently, it has been shown that physical frailty occurs more frequently in those with hepatic encephalopathy awaiting a LT (105, 106). Nevertheless, little is known regarding the ability to safely apply therapeutic interventions, such as exercise, to those with overt hepatic encephalopathy to reverse physical frailty in these patients. Whilst ammonia is known to play a central role in the development of hepatic encephalopathy, and therefore is likely to contribute also to physical frailty seen in these patients (107), further understanding of the pathophysiological links between ammonia, hepatic encephalopathy and physical frailty is needed to guide future intervention.

1.4.2.2 Ascites

Ascites occur as a result of portal hypertension and an inability to excrete sodium into urine, resulting in a positive fluid balance (108, 109). The altered architecture and raised nitric oxide levels within the liver and splanchnic and peripheral circulation leads to increased portal flow and decreased splanchnic and systemic vascular resistance, respectively (110). Consequently, the vasoconstrictor neurohumoral systems (i.e. renin-angiotensin-aldosterone, sympathetic nervous system, and antidiuretic hormone) are activated leading to retention of salt and water (110). Ascites is the most common complication of cirrhosis with 60% of patients with cirrhosis developing ascites within 10 years (111). The development of ascites can be graded from 1-3, representing the progression of ascites severity. Generally, once ascites has developed, prognosis is poor with a 50% mortality rate within three years (112, 113). Similar to hepatic encephalopathy, whilst physical frailty appears to be more prevalent in those with ascites, the exact mechanism between ascites and physical frailty is currently unknown (106).

1.4.2.3 Physical Inactivity

1.4.2.3.1 Prevalence of physical inactivity in ESLD

Physical inactivity (i.e. low involvement in physical activity) is highly prevalent in patients with chronic liver disease with studies reporting over 76% of people with cirrhosis having low involvement in physical activity (114). It is associated with increased risk of the development of liver disease (Hazard ratio (HR) 0.62, 95% confidence interval (CI) 0.53-0.73, p=0.0001) as well as liver disease progression (HR 0.58, 95% CI 0.43-0.79, p=0.009) (115). Patients with CLD have been reported to, on average, complete between 2401 and 4461 steps per day, with an

average daily step count of ≤1163 being associated with increased risk of hospitalisation and death (HR 1.9, 95% CI 1.09-3.30 and 3.46, 95% CI 1.23-9.68, respectively) (116).

1.4.2.3.2 Causes of physical inactivity in ESLD

Physical inactivity may be due to a multitude of barriers to activity in a patient with CLD including: fatigue; fluid overload; anaemia; pain; altered metabolism and disturbances in mental health (117). Yet, these prolonged periods of inactivity in CLD have a damaging effect on skeletal muscle regulation. Skeletal muscle inactivity causes muscle fibre atrophy as a result of increased muscle protein breakdown, by all four major proteolytic systems (ubiquitin proteasome pathway, caspase-3, calpain, and mitophagy), and a decreased rate of muscle protein synthesis (118). Some of these changes may be explained by reduced insulin sensitivity, increased production of reactive oxygen species (ROS) and mitochondrial dysfunction (119).

Sedentary time, the period of complete physical inactivity, is now known to be a separate variable influencing muscle physiology, resulting in reduced AMPK activation and uptake of glucose by the skeletal muscle (119). In addition, the elevated levels of circulating glucose in the plasma provide a substrate for de novo lipogenesis in adipose tissue and the liver (120). As a result, adipose tissue mass expands and intrahepatic lipids accumulate resulting in an increase in lipid export from the liver as very low density lipoprotein triacylglycerol particles and serum triacylglycerol, inducing systemic insulin resistance and consequently reducing muscle protein synthesis (120). Prolonged sedentary periods also alter mitochondrial function by increasing mitochondria DNA mutation and fission whilst reducing mitochondria biogenesis (121). These mechanisms result in reduced ATP production and increased release

of ROS, which in turn decreases activation of the P13K/AKT pathway and consequently increases muscle protein breakdown (122).

1.5 Assessment of physical frailty in chronic liver disease

Despite the recent surge of evidence, the vast majority of hepatology departments do not routinely perform objective measures of physical frailty. This may be due, in part, to a lack of clinician awareness of tools available and the benefits/limitations of such measures in patients with ESLD. Consequently, without a standardised approach to the assessment of physical frailty, inconsistent clinical decision-making and poor prioritisation of available therapies may result. The following sections will analyse and discuss the assessments available to measure physical frailty in CLD thus far.

1.5.1 Assessment of sarcopenia by muscle mass

1.5.1.1 Cross-sectional imaging

A robust index of skeletal muscle mass can be obtained using cross-sectional imaging by means of either computed tomography (CT) or magnetic resonance imaging (MRI) of the abdominal muscles at 3rd lumbar vertebrae. The cross-sectional area of the skeletal muscle is quantified using body segmentation analysis software and then normalised to the second power of height to calculate the skeletal muscle index (cm^2/m^2) (88). Although MRI and CT can be used, there is a paucity of MRI data in patients with cirrhosis and normal values are still required (88). The most commonly discussed muscle indices in the literature are total skeletal muscle index and more specifically the psoas muscle index. PMI is quick and easier to

assess than skeletal muscle index, however it is not as accurate at predicting mortality in patients (especially men) with ESLD (123).

A large systematic review of 19 studies (n=3803) by Van Vugt and colleagues showed that low muscle mass on CT-imaging was prevalent in 22% to 70% of patients selected for a LT and was associated with greater risk of death on the waiting list (HR 1.72, p=0.05) (124). Furthermore, low muscle mass resulted in increased critical care (12 versus 6 days, p=0.001) and inpatient ward (40 versus 25 days, p=0.005) length of stay, and to a lesser extent complications, including infection (124). However, due to a lack of standardised definition of sarcopenia, sex-defined cut-offs and heterogenous methods of assessment (i.e. skeletal muscle index, psoas muscle index etc.) in these studies, widespread clinical application has been challenging. Moreover, 13 of the 19 studies included patients from the same four North American Liver centres, thereby limiting their generalisability. Traditionally, skeletal muscle index-CT cut-offs were taken from oncological datasets; however, the recent formation of the North American FLEXIT (Fitness, Life Enhancement and Exercise in Liver Transplantation) Consortium has resulted in validated cut-offs for skeletal muscle index at L3 to define sarcopenia in ESLD; namely <50 cm^2/m^2 in men and <39 cm^2/m^2 in women (125). These sex-specific cut-offs of skeletal muscle index correlated with LT waiting list mortality (125, 126), but it is important to recognise both the sex and the severity of the underlying illness when applying skeletal muscle index. For example, in male patients with high MELD (>30) scores admitted with an acute deterioration that required liver transplantation, an skeletal muscle index under 48 cm^2/m^2 resulted in a 4-fold increase in post-LT mortality (127). In a separate cohort over 600 patients with cirrhosis the addition of skeletal muscle index onto the MELD (termed 'MELD-

sarcopenia') improved the predictive value of mortality, in particularly in those with a MELD <20 (126).

The most recent European Association for the Study of the Liver (EASL) Clinical Practice Guidelines in Nutrition (2019) (88) advise the use of CT to assess for low muscle mass in patients with cirrhosis and ESLD. This is achieved relatively easily for those patients being assessed for a LT, as CT is reproducible, accurate and frequently used to evaluate hepatocellular carcinoma, vasculature and biliary anatomy. However, CT is expensive, time-consuming and the repeated radiation exposure restricts its use for routine and longitudinal assessment of muscle mass.

1.5.1.2 Ultrasound Imaging

Ultrasound imaging is a simple, cheap, safe and feasible method to measure muscle mass in patients with ESLD, yet only three studies have investigated its use to date (128-130). Two studies highlighted that the iliopsoas muscle was easily detectable in 80-100% of cases, with good diagnostic accuracy for sarcopenia (area under the receiving operator characteristic (AUROC) 0.84) and acceptable intra- and inter-operator variability (128, 129). Furthermore, ultrasound defined iliopsoas muscle index (muscle area to patient height^2 ratio) significantly correlated with CT in both sexes ($r>0.90$, $p<0.0001$) (129) and was associated with increased risk of hospitalisation and mortality (HR 0.91 and 0.93, respectively) in 75 patients with decompensated cirrhosis (128). Identification of the Iliopsoas muscle was mainly limited in patients with high abdominal circumferences (128), calling into question its accuracy in patients with ESLD and morbid obesity. Alternatively, Tandon and colleagues evaluated ultrasound to measure thigh (quadricep) muscle thickness in 159 patients with cirrhosis (60%

CPS A) compared to CT-skeletal muscle index or MRI (130). Targeting the quadriceps demonstrated excellent inter-observer reliability (correlation 0.97), and when combined with body mass index (BMI) it identified sarcopenia in male and female patients almost as well as cross-sectional imaging (AUROC 0.78 and 0.89, respectively). Despite, the fact larger prospective longitudinal studies are needed, ultrasound shows promise and may play a unique future role in monitoring and assessing response to nutrition in bed-bound inpatients and those who are critically unwell.

1.5.1.3 Dual Energy X-ray Absorption (DEXA)

Dual energy x-ray absorption (DEXA) is an easy, reproducible and accurate method in the general population to analyse body composition (fat and fat-free mass), with minimal radiation exposure (131). Yet, the analysis of muscle mass using fat-free mass index (kg/m^2) in DEXA can be overestimated due to its inability to distinguish water from muscle, which is particularly problematic in patients with ascites, hydrothorax and/or peripheral fluid retention (132). Belarmino and colleagues aimed to overcome this limitation by using appendicular (arm or leg) skeletal muscle index (kg/m^2) and demonstrated no change in DEXA-appendicular skeletal muscle index before and after abdominal paracentesis (132). However, despite this Giusto and colleagues still highlighted that DEXA-appendicular skeletal muscle index only weakly correlated with skeletal muscle index-CT, all be it in only 59 patients (131). This discrepancy may be explained by the fact that DEXA-appendicular skeletal muscle index may have detected fluid retention in the lower limbs, as more recent studies have highlighted differences in the predictive accuracy of DEXA in the upper versus the lower limbs in cirrhosis. In a recent study of 429 men with cirrhosis, DEXA measures of appendicular lean mass of the upper limb were strongly associated with mortality (HR 0.27, p=0.004), whereas

measures of lower limb were not (HR 1.02, p=0.71) (133). Targeted DEXA measures of upper limb lean muscle mass may provide a safer, more accessible and quicker tool in the clinical setting of ESLD, however, larger studies are needed to validate these findings (especially in women).

1.5.1.4 Anthropometry

Mid-arm muscle circumference (MAMC, cm) is obtained by measuring the mid-arm circumference (MAC, cm) and triceps skin fold (TSF, mm); calculated MAMC = MAC – (3.14 × TSF). These measures are the quickest, simplest and most inexpensive way to assess muscle mass at the bedside or in the outpatient clinic. When performed by trained personnel, both methods have good intra- and inter-observer agreement (correlation coefficient 0.8 and 0.9, respectively). MAMC is a better predictor of mortality when comparing patients who are below the 5th percentile for muscle mass with those above (p=0.001) (134). Furthermore, in one study MAMC was a good predictor of low muscle mass when CT was used as the gold standard (AUROC 0.75 in men and 0.84 in women) (130). Therefore, MAMC can be used as a screening tool to highlight those patients with potential sarcopenia that require assessment of their physical function and targeted prehabilitation.

1.5.2 Assessment of physical function

1.5.2.1 Hand Grip Strength

Recent International Clinical Practice Guidelines (EASL, European Society of Clinical Nutrition and Metabolism (ESPEN), 2019) recommend that all patients with ESLD should undergo assessment of muscle mass and strength with MAMC and HGS, respectively (88, 135).

Measurement of HGS is a quick, simple and inexpensive method of measuring upper limb muscle strength. It is recommended that it is performed three times in the 'non-dominant' hand and the mean value compared with historical 'normal' values for women (29kg) and men (40kg). HGS is significantly lower in LT waiting list cohorts when compared to normative data for older adults (60-69 years) (median 28kg, inter quartile range [IQR] 21-27 (n=536) versus 40kg/24kg (males/females), p=<0.001) (136). Low HGS is associated with hospitalisation (median 27.7 kg [hospitalised] versus 32.7 kg [not hospitalised]) (137), low physical activity, malnutrition, hepatic encephalopathy and severe liver disease (105, 136, 138). In a multivariate analysis, Hanai and colleagues showed HGS was also associated with all-cause and liver-related mortality independent of age, aetiology of cirrhosis, development of HCC and serum sodium level (HR 0.96, P=<0.001)(139). Although this study has its limitations (*older adults [>70 years]; 49% Hepatitis C),* it is supported by another recent study by Sinclair and colleagues (140) (n=145, mixed aetiology of liver cirrhosis) who showed that with every 1kg increase in HGS, survival was increased by 6% (140). However, this study investigated male patients with liver cirrhosis only and further research is needed to establish the mortality risk, as well as cut-off points in females and all liver aetiologies.

1.5.2.2 Chair Stands

Chair stands are simple and a bedside appropriate measure of muscle function and strength. The number of chair stands (defined as rising from a seated position and returning to a seated position) completed in a set time period is recorded. Lai and colleagues found chair stands to be one of the strongest predictors of waiting list mortality when used in combination with HGS (AUROC 0.72) (136). Furthermore, those who complete less than 10 chair stands within 30 seconds had a sensitivity/specificity 73%/54% for falls (141) and those who can complete

five chair stands within 10 seconds have less chance of developing an infection (p=0.04) (137). Nevertheless, further research is needed to validate chair stands as a measure of frailty in ESLD, as well as to determine specific cut-off points for predicting clinical outcome.

1.5.2.3 Gait Speed

Gait speed is a reproducible way of measuring physical function in patients awaiting a LT (142). The participant uses a self-selected (usual pace) gait speed over a set distance (usually 2.4 to 5m). It can be used as a stand-alone test or as part of a battery of tests such as Short Physical Performance Battery (SPPB). Gait speed was found to be slower in patients listed for LT (n=350) when compared to normative data for older adults (mean gait speed: males 0.90metres/second (m/s) vs. 1.3 m/sec; females 0.98 m/sec vs. 1.2 m/sec) (142). Overall, slow gait speed is significantly associated with poorer outcomes such as higher rates of hospitalisation (p=<0.001) and risk of waitlist removal (p = 0.02) (29). Indeed, patients removed from the LT waiting list at the University of Pittsburgh Medical Centre had significantly slower gait speeds than those who remained active on the list (0.92m/s versus 1.03m/s, p<0.05). Even though statistically significant, a clinical difference of as little 0.11m/s between these patient groups questions the relevance of gait speed in isolation.

1.5.2.4 Short physical performance battery (SPPB)

The SPPB is an inexpensive and efficient assessment tool designed to measure functional status and physical performance. It is calculated from three components: time to complete five chair stands; time to walk 4m and balance testing. Each component is scored out of four, with the scores combined to give a total score out of 12 (range 0-12) (143), with the higher scores representing the best physical status.

SPPB scores are significantly lower in older compared to younger LT candidates (median 10 (9-11) versus 11 (9-12); p=0.01) (144). An SPPB score of ≤9 predicts a higher risk of waiting list mortality in both young (HR 1.77, p=0.03) and older (HR 2.70; p=0.03) patients (144). However, studies have highlighted that the majority (68%) of LT waiting list patients score ≥10 (144) and while these may have a lower risk of waiting list mortality, functional decline on the waiting list occurs at a median rate of 0.16 SPPB points every 3 months (145). This implies that a significant proportion of patients may deteriorate below a SPPB of 10 whilst on the waiting list; especially those with the longer waiting times. Early identification of those at risk of functional decline remains a challenge, but the Functional Assessment in Liver Transplantation (FraiLT) study data highlights that tools such as SPPB may be useful in identifying those most in need of prehabilitation (146). Whether or not SPPB can be reliably used as a serial measure of response to prehabilitation remains to be seen. Colleagues and I found a ceiling effect of SPPB scores (i.e. maximised to 12/12) in 18 patients who received 12-weeks of home-based exercise whilst on the LT waiting list (117). Although a small sample size, our study highlights that additional functional gains with prehabilitation may be missed using SPPB alone, especially in those who have a high score at baseline.

1.5.2.5 Liver Frailty Index (LFI)

The LFI is a composite metric of three performance-based measures: hand grip strength (HGS), time to do 5 chair stands (seconds) and time holding 3 balance positions (feet side by side, semi-tandem and tandem) to objectively assess physical frailty in ambulatory patients with ESLD (136). The LFI score can be calculated using an on-line calculator (available at: http://liverfrailtyindex.ucsf.edu) with patient physical frailty categorised as robust, pre-frail

and frail according to their index (index = <3.2 (robust), 3.2-4.5 (pre-frail), >4.5 (frail)). Most recently, optimal cut-offs of frailty have been developed in a multi-centre US study of 1405 patients to predict mortality on the waiting list after 3-months (LFI >4.4) and 6-12 months (LFI 4.2) (147). Overall, the LFI is a reliable test (correlation coefficient 0.93) and is well validated in cirrhosis (106), whereas it has been investigated to a lesser extent in patients without cirrhosis (148). Importantly, it is a liver disease-specific, continuous variable (i.e. no ceiling or floor effect) that is inexpensive, quick to complete (3-5 minutes) and requires minimal space and staff training, making it a useful and practical tool for measuring physical frailty in the clinical setting.

In a study of 529 participants a higher LFI (i.e. greater degree of frailty) pre-LT was significantly associated with waiting list mortality (HR 2.9, p<0.001) and length of stay post-LT (9 vs 7 days, p=0.004) (146, 149). Furthermore, LFI was shown to predict physical recovery post-LT with those who are categorised as frail pre-LT being less likely to return to a "robust" state within 12 months of transplantation (149). LFI is the best studied outpatient measure to date in the setting of liver transplantation, however there is a pressing need to validate it outside of the United States, in hospitalised inpatients, and in the acutely unwell (i.e. acute-on-chronic liver failure).

1.5.2.6 Six-minute walk test (6MWT)

The 6MWT is a self-paced field walking test conducted under controlled conditions and is a reliable and valid measure of exercise tolerance in various patient populations (150, 151). The distance walked in six minutes (6MWD) is 27% shorter in patients with cirrhosis than in normal controls and is further reduced in patients with ESLD and advancing Child-Pugh classification

(152). A reduced 6MWD predicts LT waiting list mortality (150-154), with those scoring under 250m twice as likely to die prior to a LT (154). Every 100m decrease in the 6MWD represents an almost 50% increase in waitlist mortality, independent of liver disease severity (based on MELD).

The test is inexpensive and simple to administer, however a number of issues may limit its practical application. It requires a 30 metre level indoor walking course and the course layout and degree of patient encouragement must be standardised, as they significantly affect the distance walked (155). Strong evidence of a learning effect (i.e. patient becomes more familiar with the test) has been seen in studies using repeated 6MWT, and this may complicate the clinical interpretation of changes in test results over time (156). The learning effect may be reduced by performing two tests and recording the best result at baseline assessment.

1.5.3 Assessment of aerobic capacity

Reduced aerobic capacity is a fundamental component of frailty, reflecting limited reserve capacity of multiple organ systems and contributing to low habitual activity levels and slow walking speed (48, 157-159). In patients with ESLD, aerobic exercise capacity is substantially poorer than general population norms, and in turn is associated with poorer overall survival (153, 160)

1.5.3.1 Cardiopulmonary exercise testing (CPET)

Cardiopulmonary exercise testing (CPET) is the gold standard assessment of aerobic exercise capacity. It directly assesses gas exchange, work, heart rate and rhythm, and blood pressure during intense exercise. In a small prospective UK study of patients undergoing assessment

for liver transplantation, Prentis and colleagues demonstrated that an oxygen consumption at the anaerobic threshold (AT) of less than 9ml/kg/min was a good discriminator of 90-day postoperative mortality, with 90.7% sensitivity and 83.3% specificity (161). It is important to not over interpret the AT cut-off in this study due to the small sample size of 60 patients and the fact there were only 6 reported deaths. In the largest retrospective study to date (n=399), Bernal and colleagues demonstrated that low AT was associated with reduced survival and increased postoperative hospitalisation for patients undergoing LT (160). Furthermore, they found that low AT and low peak oxygen consumption were associated with reduced one-year survival among patients who were assessed for, but did not undergo LT.

In 2016, Ney and colleagues performed a 7 study (1107 patient) meta-analysis in patients awaiting (3 studies) or post LT (4 studies) (162). The majority of these studies were retrospective and only included those deemed fit enough for a LT (i.e. selection bias). Overall, they found that AT was the CPET variable most consistently associated with LT outcomes, with mean differences of 2.0ml/kg/min between survivors and non-survivors. In contrast with field walking tests, measurement of the AT does not require maximal patient effort and is less likely to be confounded by volitional factors. CPET may also provide data to support a diagnosis of cardiovascular, respiratory or metabolic disease in patients with limited exercise capacity. However, the use of CPET in ESLD is limited by the requirement for costly equipment, specifically trained staff and the lack of robust AT cut-offs for predicting mortality due to study heterogeneity (162).

1.5.3.2 Duke activity status index (DASI)

The Duke Activity Status Index (DASI) is a 12-item self-reported assessment of functional capacity that requires minimal time to complete (163). It provides prognostic information in a variety of chronic diseases and can be used as an index of disease progression over time (164-166). The DASI has been shown to be a useful predictor of adverse outcomes (death, myocardial infarction) after major non-cardiac surgery (167), over and above that of CPET and serological tests (i.e. NT Pro-BNP). However, there has been no published data of DASI in patients with ESLD or LT, but based on the recent findings in major non-cardiac surgery and its ease/cost-savings of completion, validation of DASI should be sought.

1.5.4 Assessment of functional independence

1.5.4.1 Activities of daily living (ADL)

Physical disability, as indicated by impaired ADLs (bathing, dressing, toileting, continence and feeding) or IADLs (using a telephone, shopping, food preparation, housekeeping, doing laundry, transportation, managing finances and managing medications), is more prevalent among older people with cirrhosis than in those without CLD (168). Forty percent of patients with ESLD have impairment of at least one IADL, and in this group physical disability is associated with adverse outcomes. Specifically, impairments of toileting, transferring, housekeeping and laundry have been found to associate with mortality on the LT waiting list (169). Liver transplantation appears to reduce disability among recipients, with an improvement in ADLs seen at 6 and 12 months post-LT (170).

1.5.4.2 Karnofsky Performance Status Scale (KPS):

Reduced performance status and low levels of habitual activity are key components of the frailty construct. A number of scales have been developed to quantify patient and clinician assessment of performance status, but only the Karnofsky Performance Status Scale (KPS) has been utilised in the setting of ESLD and LT. Developed more than 70 years ago as a measure of functional independence for patients with cancer, the KPS is a unidimensional clinician-reported measure ranging from zero (death) to 100 (no limitation). It may aid prognosis in a variety of chronic disease states, following acute medical admission and predicting decline in older outpatients (171-173). A large retrospective US transplant registration series (>70,000 patients) has demonstrated an association between a low KPS and death among patients awaiting LT (174). KPS tends to decline over time as patients await a LT, and then to improve in the post-LT period. Furthermore, recipients with lower KPS or a failure to improve KPS post-LT have poorer graft and patient survival (175). The KPS also improves prediction of death in patients with ESLD and whom are within 3-months of discharge from hospital (176). The effect on clinical outcomes of utilising the KPS to prioritise those patients most in need of early follow-up, closer monitoring and targeted prehabilitation has not been studied.

1.6 Physical activity and exercise to improve physical frailty in chronic liver disease

Whilst often used interchangeably, physical activity and exercise are not the same intervention. Physical activity is any bodily movement produced by skeletal muscles that increases energy expenditure, whereas exercise is a subset of physical activity which is planned, structured, and repetitive with the aim of maintaining and/or improving physical

fitness (177). Therefore, for the purpose of this section, these two types of intervention are discussed separately.

1.6.1 Physical activity to improve physical frailty in CLD

Only one study to date has investigated the use of a physical activity intervention to improve measures of physical frailty in patients with CLD (178). In a small (n=17) randomised control trial (RCT) Chen et al. asked participants to wear an accelerometer device for 12-weeks. Following a 2-week run in time to assess baseline physical activity levels, participants met with a member of the research team to receive advice on exercise and physical training, mainly increasing daily steps, alongside some brief behavioural counselling. Every 2-weeks investigators reviewed and met participants to set new goals with the aim to increase biweekly steps/day by 500. Whilst there was no within group significant change in daily steps, VO_2peak or 6MWT, there were significant between group differences in daily steps (+2627steps, p=0.001) and 6MWT (+151m, p=0.03). The lack of significant improvement seen within the intervention group may be attributed to the lack of an appropriate intensity needed to stimulate physiological change. The significant between group differences were seen mainly due to a decline in daily steps and 6MWT scores seen in the control group. This indicates that whilst physical activity interventions may not be enough to significantly improve physical frailty, they may play a role in reducing decline, which in itself holds benefit for preventing poor clinical outcomes.

1.6.2 Exercise to improve physical frailty in CLD: a review of the literature

Exercise is a well-recognised therapeutic intervention to improve physical frailty in a variety of chronic diseases (i.e. cardiovascular, respiratory, metabolic) (179), as well as in other non-

liver solid organ transplants (i.e. heart, lung, kidney) (180). Whilst evidence for the clinical use of exercise in CLD is building, there remains a lag behind other disease cohorts. This is, in part, explained by the historical concerns that exercise may increase portal pressures and subsequently increase the risk of variceal haemorrhage (181). Furthermore, the unique obstacles to exercise that accompany CLD namely, ascites, hepatic encephalopathy, fatigue/lethargy, may hinder exercise participation.

In recent years, the safety of supervised and home-based unsupervised exercise in CLD has been proven (182-184). Yet, there remains a lack of consensus for exercise guidance. Fourteen studies to date have investigated the impact of exercise therapy in patients with compensated (n=9) (**Table 1.4**) or decompensated (n=5) (**Table 1.5**) cirrhosis; including nine RCT, four observational cohort studies and a case study (178, 183-195). The majority of these studies were small (median 21, interquartile range (IQR) 17-45), and focussed on supervised (n=9 supervised/part-supervised), hospital-based aerobic exercise interventions (i.e. cycle ergometer). There was a male predominance throughout (257/394, 65%), with the median age at recruitment being 56.5 years (IQR 55-62) and the majority of studies (9/14) were in compensated cirrhosis. Therefore, results may not be applicable to younger female patients and those with more advanced liver cirrhosis (i.e. CPS C), the latter of which one could argue are in most need of physical optimisation.

Frequency, intensity, time and type of exercise programmes delivered varied between studies. Initially, exercise programmes mainly consisted of supervised aerobic exercise (i.e.

cycle ergometer, walking and step-ups) (178, 186-190, 193, 194), whilst some went on to also include strengthening resistance exercises (i.e. weights and/or bodyweight circuits) (183, 191, 195). The intensity of exercise varied from the recovery training zone (defined as Borg rate of perceived exertion (RPE) 10-12 i.e. breathing is somewhat hard) to anaerobic training (Borg RPE 14-15 i.e. hard to breath) (196) with a variable frequency of delivery of 1-4 hours per week. More recently, there has been a focus upon resistance only based exercise programmes (184, 185, 192), yet only the compensated cohort adhered to such a programme (185). A lack of standardised approach to exercise in CLD, small sample sizes and variance in disease severity limits any accurate comparisons between these studies, however, themes and knowledge can be drawn within the domains of physical frailty to help guide clinical practice and future designs of RCTs.

1.6.2.1 Impact of exercise on muscle mass, strength and function

Muscle mass and strength decline over time in patients with CLD and are associated with loss of function (197), poor clinical outcomes (198), and increased healthcare costs (199). Consequently, interventions to increase muscle mass and strength could improve health-related outcomes and costs in patients with CLD.

Table 1.2 A summary of clinical studies of exercise interventions to improve physical frailty in compensated cirrhosis

Study	Design	Sample size	MELD	CPS-A (%A)	Exercise type	Duration (wks)	Adherence (%)	Muscle mass	Muscle strength & function	Aerobic capacity
Aamman et al. 2020	RCT	39 (19 control)	11	50	Supervised, individualised resistance	12	"High"	Quad CSA ↑ 5.2cm^2*, MAMA ↑ 9.8cm*	Peak knee extensor torque ↑ (15N-m)*	-
Berzigotti et al. 2017	Observational	50	6	92	Supervised aerobic, resistance	16	88	-	-	VO_2peak ↑ 4.4mL/kg/min
Hallsworth et al. 2016	Case study	1	9	100	Unsupervised aerobic	64	-	-	-	VO_2peak ↑ 8.6mL/kg/min AT ↑ 3.2mL/kg/min
Kruger et al. 2018	RCT	40	9	70	Part-supervised aerobic	8	55	TC ↑ 1.8cm, no sig. change in control group	-	VO_2 peak ↑ 1.7mL/kg/min 6MWT: ↑ 24m No change in control group
Nishida et al. 2016	Observational	6		100	Part-supervised aerobic	52	100	-	-	METs: ↑1.5, no change in daily physical activity
Roman et al. 2014	RCT	17	9	82	Supervised aerobic	12	17	Lower TC ↑5cm*, no change in control group	-	6MWT: ↑ 80m* 2MST: ↑ 50steps* No change in control group
Roman et al. 2016	RCT	23	9	100	Supervised aerobic	12	6	Lean BM and LM: ↑ 1.05kg** & 0.34kg No sig. change in either in control group	-	VAT[b] time: ↑ +1.5min**, no change in control group VO_2peak: no sig. change in either group
Sirisunhirun et al. 2022	RCT	40	8	100	Unsupervised, resistance	12	75	No sig. change in RF feather index		6MWT: ↑ 18.8m
Zenith et al. 2016	RCT	19	11	74	Supervised aerobic	8	-	TC ↑ 1.24cm*		VO_2peak: ↑ 5.3mL/kg/min** compared to control group 6MWT: ↑ 23.5m*

Abbreviations: AT = anaerobic threshold, BM = body mass, cm = centimeters, CPS-A = Child-Pugh Score A, CSA = cross-sectional area, kg = kilogram, LM = leg mass, MAMA = mid-arm muscle area, m = metres; METs = metabolic equivalents, min = minute, mL = millilitres, N-m = newton metre, RCT = randomised control trial, RF = rectus femoris, sig. = significant, TC = thigh circumference, VAT[b] = ventilatory anaerobic threshold time, VO_2peak = peak oxygen uptake, wks = weeks, 2MST = two-minute step test, 6MWT = six-minute walk test

*significant change

Table 1.3 A summary of clinical studies of physical activity and exercise interventions to improve physical frailty in decompensated cirrhosis

Study	Design	Sample size	MELD	CPS-A (%A)	Type	Duration (wks)	Adherence (%)	Muscle mass	Muscle strength & function	Aerobic capacity
Chen et al. 2020	RCT	17	17	0	Remotely monitored HB-PAP (step/activity programme)	12	-	CT-based PMI: ↑0.7 intervention SMI: no change	Daily steps: between group difference (2627 steps/day)*, no sig. change in intervention group	VO_2peak: no sig. change 6MWT: between group difference +151m* No sig. change in intervention group
Debette-Gratien et al. 2015	Observational	8	13	63	Supervised aerobic, resistance	12	-	-	Max power ↑ 13W* IQS ↑ 7W*	VO_2peak: max power ↑ 1.7mL/kg/min 6MWT: ↑ 40m*
Lai et al. 2020	RCT	83	14^	36	Home-based resistance	12	14	-	LFI: ↓ 0.2 (p=0.65)	-
Morkane et al. 2019	RCT	33	13	-	Supervised aerobic	12	94	-	-	VO_2peak ↑2.4mL/kg/min*
Williams et al. 2019	Observational	18	18	-	Home-based aerobic and resistance	12	82-90	-	SPPB ↑ 2.5* Daily steps ↑2700steps	ISWT: ↑ 210m*

Abbreviations: CPS = Child-Pugh Score; CT = computed tomography; HB-PAP = home-based physical activity programme, IQS = isometric quadriceps strength; ISWT = incremental shuttle walk test; kg = kilogram; LFI = liver frailty index; MELD = Model for End-Stage Liver Disease; m = metres; max = maximum; min = minute; mL = millilitres; PMI = psoas muscle mass index; RCT = randomised control trial; sig. = significant; SMI = skeletal muscle mass index; SPPB = short physical performance battery; VO_2peak = peak oxygen uptake; W = watts; wks=weeks; 6MWT = six-minute walk test

*significant change

^MELD-Na

Eight studies, to date, have measured change in muscle mass (178, 184, 185, 188-190, 194) and/or muscle strength (185, 191), but with inconsistent results. This may be due to the heterogeneity of intensity, type and duration of the reported exercise interventions. Debette-Graiten and colleagues instructed 13 participants with decompensated cirrhosis to complete twice-weekly cycle ergometry and muscular strengthening exercises on a weight bench (i.e. series of 8 repetitions at 70%-80% of their maximal strength) for 12-weeks (191), which yielded significant improvements in isometric (mean difference (MD) +7 watts, $p<0.01$) and maximal (MD +13 watts, $p<0.05$) quadriceps strength. Similarly, Aamman et al. reported a significant improvement in peak knee extensor strength (MD +15N-m, $p<0.001$), quadriceps cross-sectional area (MD +5cm^2, $p<0.001$), and mid-arm muscle area (MD +9.8cm2, $p<0.01$) following a resistance-based exercise programme consisting of 1-3 sets of 8-10 repetitions of each exercise at a moderate level where the last two repetitions were deemed challenging. Yet, despite also using a resistance-based exercise intervention, Sirisunhirun et al. did not see significant changes in rectus femoris thickness. This may be due to the intensity of the intervention. For example Sirisunhirun et al. maintained exercise repetitions at 15 throughout the 12-week intervention. Without progression of exercise intensity/muscle loading then muscle hypertrophy is unlikely to be induced.

In contrast, despite no use of resistance exercise, Zenith et al. reported significant improvement in thigh circumferences (MD +1.8cm, $p=0.001$) in compensated cirrhosis patients (190). This is likely attributed to the dominant use of the lower limbs during the cycle ergometry performed in this study (**Table 1.4**). In all of these studies, it is unclear if changes to muscle mass and strength had an impact on patient's level of function. Two studies investigated change in function in patients awaiting a LT. Colleagues and I found a significant

improvement in SPPB scores (MD +2.0 points, p=0.02) as well as a 44% increase in patients reporting no problems with mobility (Euroquol-5 dimension-5 levels quality of life questionnaire) following our 6-week home-based combined aerobic (walking) and body-weight resistance exercise intervention (183). Lai et al. also found improvements in function with a 0.2 mean decrease in LFI scores following 12-weeks of a 30 minute strengthening programme completed three times a week via video, though this was not significant. Difference in significance of outcomes may be attributed to differences in adherence, with 88-92% adherence reported in my study, compared to 14% in the study by Lai and colleagues. Improvements in function may have a considerable positive impact on social care requirements as well as patient and carer quality of life. Thus, inclusion of functional measures such as the LFI and SPPB should be considered in future studies investigating the effect of exercise in CLD.

One important consideration for the development of muscle mass and strength is the importance of adequate dietary protein intake and its timing in relation to exercise. In general, specific dietary protocols before and after exercise interventions were poorly documented in the published studies, with the majority focused on vitamin supplementation (187, 189). Berzigotti et al., in their Spanish study of 50 obese patients with cirrhosis, aimed to maintain protein intake at 20%-25% of participant total intake, in order to reduce the risk of muscle wasting with exercise, whilst trying to achieve fat mass loss with calorific restrictions. Despite including resistance exercises (1-hour duration weekly for 4 months) there were no significant improvements, but overall muscle mass was maintained, which by avoiding natural deterioration (as seen in controls), may have had a positive impact on clinical outcomes with longer follow-up. The intensity of resistance exercise was not reported in this

study, making it difficult to compare to other studies, but it did highlight that “safe” weight loss is possible in patients with compensated cirrhosis and portal hypertension without detrimental muscle loss. Certainly, further research is required to fully understand the optimal resistance exercise programme and corresponding nutritional requirements in CLD (either side of exercise).

1.6.2.2 Impact of exercise on aerobic capacity

A minimum of twice weekly supervised aerobic exercise sessions (i.e. treadmill or cycle ergometer) for 2-3 month duration has been shown to significantly improve VO_2 peak in patients with cirrhosis (+1.7 to 5.3 mL/kg/min, $p<0.05$) (190, 194). In contrast, others have shown no effect of aerobic exercise in this setting (+1.6 mL/kg/min, $p<0.05$) (188). Discrepancies between studies may be due to the various intensities of the exercise intervention. Studies which reported significant improvements in aerobic capacity required the participants to work 60-80% of their maximal heart rate or VO_2peak (190, 191, 194). In comparison the negative study (188) reported that participants only worked to ‘patient tolerance’, meaning that participants may not have exerted themselves to an exercise intensity in order to elicit physiological change. Patients with CLD notoriously have lower exercise tolerance levels (64) and often fatigue early (166) making them unlikely to push themselves without professional guidance. Therefore, future studies should consider exercise programmes with prescribed exercise training intensities, rather than using self-reported tolerance.

Additionally, it has been reported that an improvement in VO_2peak of 3.5mL/kg/min is needed to increase survival (200). However, these data are limited to male patients with

various forms of cardiovascular disease. It remains unknown whether an improvement of 1.7mL/kg/min VO_2peak is clinically meaningful in CLD. Morkane et al. reported that, while the intervention group increased their VO_2peak by +2.3mL/kg/min following a 6-week thrice-weekly cycle ergometry exercise, the control group decreased their VO_2peak by 1.9mL/kg/min (193). The latter deterioration is not surprising in light of the fact that all participants had progressive decompensated liver disease, requiring a LT. Therefore, future research should not only question what the minimal improvement in aerobic capacity needed in CLD, but whether the observed downward trajectory seen in these patients can be slowed and/or prevented with exercise.

Over half (64%) of the aerobic exercise interventions utilised expensive equipment such as treadmills and cycle ergometers. Although these modalities help guide training intensity and progression, the widespread applicability of such approach is limited, especially in health services with restricted resources. Moreover, accessibility to such equipment for patients beyond the realms of a research study may be limited, thus creating barriers to long-term lifestyle and exercise changes for patients with CLD. In light of this, Nishida et al in 2017 asked six female patients with compensated cirrhosis to undertake 140 minutes of bench step-ups per week for 12 months at home (187). The authors reported significant improvements in metabolic equivalents (METs) of task, which are objective ratios of the work metabolic rate to the resting metabolic rate (resting equals 1 MET). Although encouraging, the study sample size was small, single gender and the exercise was one-dimensional. Whether this approach would be efficacious and adhered to in a wider, more heterogeneous population remains to be seen.

Walking is another accessible, effective and low cost form of exercise (201). Evidence has shown that increasing your daily steps can have a positive impact on public health (202). Chen et al. demonstrated an improvement in 6MWT by a distance of +59m (p=.05) following a daily step programme (178). Whether this improvement is due to the intervention or to a "learning effect" is difficult to discern, as the study didn't report whether it had completed repeat baseline assessments of the 6MWT (203). However, our home-based study also found significant improvements in average daily steps and incremental shuttle walk test (ISWT) (p<.01 for both outcomes) following a 12-week exercise intervention consisting of walking and body-weight resistance exercises (2-3-times weekly). These findings demonstrate that costly equipment may not be required to produce positive improvements in aerobic capacity and function for patients with CLD.

1.6.2.3 Adherence to exercise

Adherence to exercise interventions in CLD is variable between studies (6%-100%), therefore the true impact of exercise is unknown. Kruger et al, reported larger improvements in VO_2 peak (+2.9 mL/kg/min vs + 1.7 mL/kg/min) in those that complied with the intervention, suggesting that adherence to exercise is key to improving outcomes (194). However, several barriers to exercise (i.e. social economic status, age, time commitment, social support etc) have been reported in healthy populations (204) and these barriers are amplified further in patients with CLD who have numerous additional barriers (**Figure 1.3**). It would seem plausible that learning how to overcome these barriers is essential to maintain adherence to exercise interventions, yet the method in which to achieve this remains a challenge.

At present, adherence to supervised and home-based exercise programmes is similar and both have their pros and cons. Supervised programmes provide structure, camaraderie and access to regular health care professional support, yet can be difficult for patients to adhere to due to cost/time of travel and fluctuating health status. Unsupervised, home-based exercise provides flexibility, limits patient travel and promotes independent lifestyle changes in the patient's own environment (205). However, despite weekly telephone support and fortnightly observations, the overall adherence rate in the home-based exercise study conducted by Kruger et al was low (55%). Similarly, although my study found high adherence rates in the first 6 weeks of a home-based exercise intervention (92%), adherence dropped to 78% once telephone support ceased (183). Therefore, regular contact with a health care professional is likely needed to promote adherence, with specific focus on patients' motivations and psycho-behavioural barriers towards exercise. Further research should look to combine exercise with behavioural interventions to optimise adherence to exercise interventions at home.

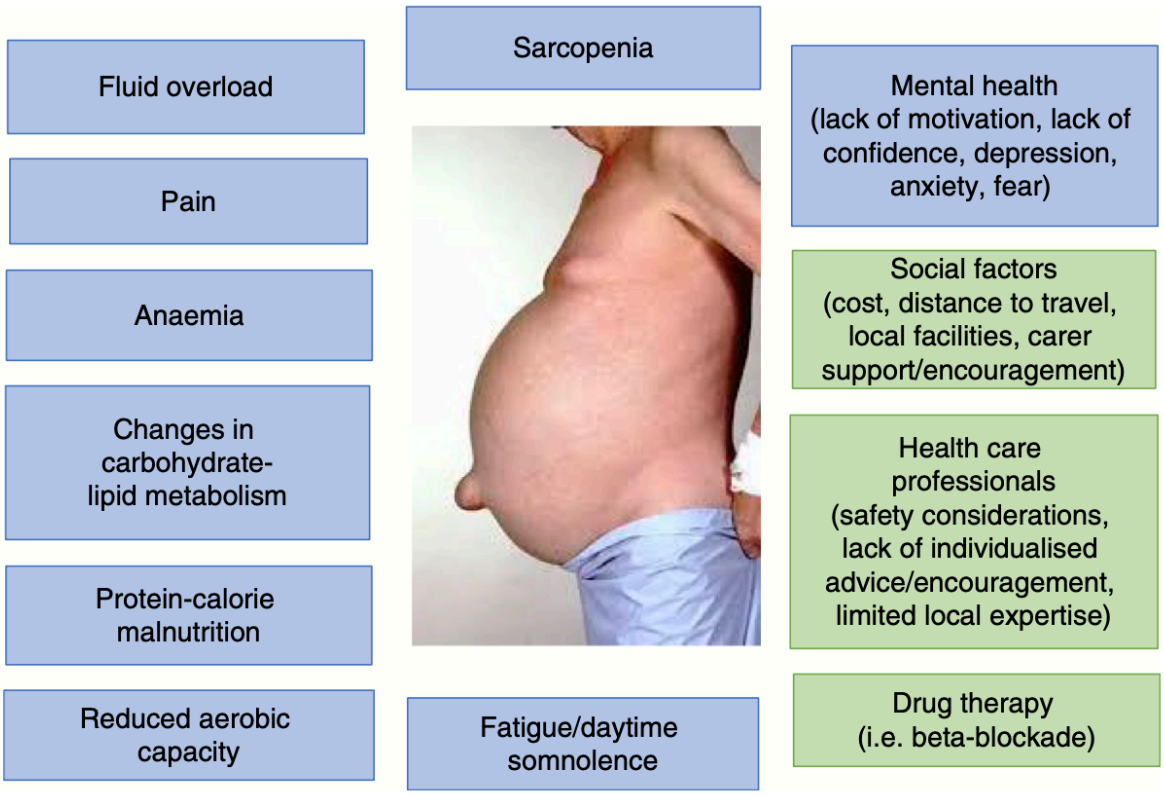


Figure 1.3 Barriers to exercise in CLD – *original figure* (206)

1.7 Assessment of physical activity

To date, a lack of robust large RCTs, heterogeneity of physical activity/exercise-based interventions and variability in adherence hinders translation of effective interventions into clinical practice. A better understanding of baseline habitual physical activity profiles would help guide future physical activity advice/exercise interventions. The gold standard method for measuring energy expenditure (i.e. physical activity) is the double labelled water method (207). However, it is rarely used in research studies due to it being time-intensive and comes with high cost and subject burden (208, 209), making it challenging to apply to large population studies. Therefore, a wide variety of alternative methods have been developed. These methods of physical activity assessment can be categorised into either; self-reported or device-based measures.

1.7.1 Self-reported measures of physical activity

Self-reported questionnaires, which identify dimensions (frequency, intensity time, type) and domains (i.e. occupational, transportation, leisure, household/domestic/self-care) of physical activity (210) are widely used in research. They are the most common method of assessing physical activity in clinical settings (211) and in large epidemiological studies, mainly due to their simplicity, low cost and ease of use (212). As such, they form the basis of the current UK Government, and World Health Organisation (WHO) physical activity guidelines (213, 214).

Within CLD, the most frequently used physical activity questionnaire is the International Physical activity Questionnaire (IPAQ) (215, 216) which has been shown to have reliability and validity across 12-countries (217). The IPAQ, which comes in a short (four generic items) and long (five domains) format, asks a series of questions about the minutes per day and days per week spent doing activities of moderate to vigorous activities as well as time spent walking or sitting in the last seven days. From this, total weekly METs are calculated and the participant is categorised into inactive (<600 METs), lightly active (600-3000 METs) or highly active (>3000 METs). However, like all self-reported questionnaires, lack of precision, overestimation and underestimation of activity, a tendency for participants to provide socially desirable results and the production of unreliable data in several populations limits its use (212, 218-220).

1.7.2 Device-based measures of physical activity

Device-based measures of physical activity record the frequency and duration of movement (211) and come in the form of pedometers, heart rate monitors and accelerometers (210). Pedometers are designed to measure walking behaviour, whereby a motion sensor is typically

worn at the hip to record movement during regular gait cycles (221). They are relatively cheap and simple to use with volume of activity being recorded in the form of steps per day (210). However, due to uniaxial data capture, they are unable to pick up on intensity of movement or the temporal characteristics of the activity pattern.

Heart rate monitors do provide information on intensity of movement and temporal characteristics. Specifically, they measure the physiological stress activity being placed on the body, making them good measures for exercise testing, and/or providing information on the dose-response to exercise (210). However, heart rate monitors are more accurately used to measure moderate-vigorous physical activity (MVPA) rather than light/inactive activity (222). This is due to the fact that heart rate can be influenced by other factors such as emotion, environment (i.e. temperature) and individual characteristics (208, 223). Individuals spend a large proportion of their day either inactive or in light intensity activity. Therefore, the effectiveness of heart rate monitors to measure 24 hour physical activity is limited.

Over recent years there has been a rapid progression in the development of accelerometers, with a publication rate increasing from 600 in 2012 and 2013 (224) to over 1500 every year since 2016. The latest research-grade models from GENEActiv, ActiGraph and Axivity are able to measure both volume and intensity of movement. All three models have been used in multiple large surveys completed by the UK Biobank and United States National Health and Nutrition Examination Survey (NHANES) (225, 226), due to their ability to measure equivalent acceleration in SI units. This, along with the development of open-source data resources, such as GGIR, facilitates transparency in data generation, and enables aggregation of data onto very large multinational databases (220). Furthermore, comparisons across populations in

relation to; prevalence of physical activity, the dose response between activity and health, and the identification of factors that affect these associations can be made (220).

1.7.3 Moving beyond cut-points

Historically, acceleration data yielded from these accelerometers has been expressed as average daily activity and/or time spent in specific intensity categories (i.e. sedentary, light, and MVPA). Whilst this method is relatively straightforward to complete, and an improvement on the older propriety count method, there are several constraints. Firstly, there are numerous cut-points available, meaning that results differ depending on the cut-point that has been used (227). Secondly, prior to analysis, data are collapsed into these set categories, meaning that data cannot be changed to be compared to another dataset using different cut-points (224). Finally, time spent inactive and in moderate-vigorous intensity are usually highly correlated, suggesting that little unique information is obtained from these measures (228).

To overcome these challenges, researchers within the field have suggested using overall activity, defined as average acceleration over a 24-hour period. Average acceleration is a directly measured element in all of the three research-grade accelerometers (i.e. GENEActiv, Axivity, ActiGraph), does not rely on population specific calibration protocols, and subsequently can be compared across all studies and populations (228). Furthermore, when worn on the non-dominant wrist, average acceleration is equivalent at light and sedentary intensities across all three devices. This equivalence is maintained at MVPA for GENEActic and Axivity, but is approximately 10% lower when using the ActiGraph and should be considered when comparing data between devices (229).

However, average acceleration only measures the overall activity of the day and does not provide detail on the distribution of intensity of that activity. For example, total activity may be equal in the following two scenarios: (a) substantial periods of time spent at MVPA with a large volume of sedentary time, and (b) large periods of time spent at light intensity, with relatively little MVPA (228). A more thorough description is detailed in Chapter 3. In short, the intensity gradient provides detail on the distribution of intensity over the course of the 24 hour physical activity profile. Therefore, when investigated together, average acceleration and intensity gradient can provide a balanced view of the 24 hour physical activity profile. This enables researchers to evaluate the relative contribution of intensity and volume of activity for a variety of health outcomes (228, 230). However, these measures are not always easily interpreted. To ensure these findings of average acceleration and intensity gradient are able to be translated into meaningful activities such as slow and brisk walking, as well as conveyance of findings into public health messages, MX metrics were developed. MX metrics, the acceleration above which the most active X minutes are accumulated, are able to translate the 24-hour physical activity profile (i.e. average acceleration and intensity gradient) onto radar plots whereby between group, activities relative to percentiles and information on meeting guidelines can be seen (229).

1.8 Overview and aims of the thesis

The presence and severity of CLD is a growing public health problem which evokes significant healthcare burden. Physical frailty, driven by intra and extra hepatic causes, has emerged as a key complication of ESLD, which is having a substantial impact upon clinical outcomes. Whilst there appears to be a robust argument for using the LFI to assess physical frailty in

North America, it is not validated within the UK and lacks accountability for alterations in aerobic capacity. Furthermore, despite the growing popularity of physical activity and exercise-based interventions to reverse physical frailty in ESLD, success of these interventions is variable. Further understanding of habitual physical activity levels, and their impact on physical frailty, is needed to guide future large exercise-based RCTs in patients with CLD.

Therefore, the studies presented in this thesis aim to:

1. **Identify the prevalence and predictors of physical frailty in ESLD.**
2. **Validate the measures of physical frailty, the LFI and DASI, within a UK-based cohort**
3. **Provide an in depth description of habitual physical activity profiles of those with ESLD and compare them to those of healthy controls**
4. **Describe the factors that influence physical activity profiles in ESLD**
5. **Provide further understanding into how different physical activity profiles can influence physical frailty in ESLD**

The main research gaps and objectives for each results Chapter are provided in **Table 1.4** Overview of results chapters.

Table 1.4 Overview of results chapters

Chapter	Research gaps	Research objective
Two	Physical frailty is common in ESLD, yet evidence is limited to cohorts outside of the UK. Prevalence and factors associated with physical frailty within the UK are currently unknown. Furthermore, there is no consensus for the assessment of physical frailty within the UK. Whilst the LFI is widely researched and used in North America, it is not validated within the UK. The DASI is an alternative tool to time and resource burdensome measures such as CPET and 6MWT, and has shown promise in predicting aerobic capacity in patients awaiting non-cardiac major surgery, but has not been validated within liver disease.	1. Identify the prevalence of, and factors associated with, physical frailty in a UK-based LT centre 2. Validate the LFI and DASI in a UK-based cohort being assessed for a LT 3. Validation of DASI within CLD
Four	There is limited objective data for habitual physical activity profiles of patients with ESLD. Data available is limited to traditional cut-points which only provide information for small proportions of the day. Furthermore, these data are not comparable to other cohorts due to the use of propriety data sets.	1. Describe the physical activity profile in relation to traditional cut-points (sedentary, light, moderate, vigorous activity) and compare this to healthy controls 2. Describe the 24 hour physical activity profile (volume (average acceleration) and intensity distribution (intensity gradient)) for patients with ESLD and compare these to healthy controls 3. Translate 24 hour profiles into visual illustrations of every day activity by comparing MX metrics of the 24 hour profile for ESLD and HC on radar plots
Five	There is no research that investigates factors that influence objective physical activity data. Furthermore, there is no research on how physical frailty is associated with the physical activity profile in terms of volume of physical activity and intensity distribution.	1. Identify factors which influence the physical activity profile in terms of the volume of activity (average acceleration) and the intensity distribution (intensity gradient) 2. Determine the relative importance of volume of activity and intensity distribution for physical frailty in patients with ESLD

CHAPTER 2:

SIMPLE MEASURES OF PHYSICAL FRAILTY AND FUNCTIONAL CAPACITY PREDICT WAITING LIST AND OVERALL MORTALITY IN PATIENTS ASSESSED FOR A LIVER TRANSPLANT: A UK PROSPECTIVE COHORT STUDY

2.1 Introduction

Patients listed for liver transplantation (LT) in the current era tend to be sicker, medically more complex and are more often described as 'frail' (231). This is largely due to an ageing population, increased prevalence of metabolic-related liver disease (i.e. diabetes, obesity) and worsening degree of liver disease severity at the time of LT assessment. Frailty is a multidimensional clinical state of decreased physiological reserve and increased vulnerability to health stressors, including surgery (232). Physical frailty, in particular, refers to the functional ability (i.e. functional performance, aerobic capacity and disability) of a patient and is the most widely investigated component of frailty within the LT field (231). Physical frailty is highly prevalent in end-stage liver disease (ESLD) and is an independent predictor of adverse clinical outcomes in North America (233). Despite this, in Europe objective and reproducible assessments of physical frailty are scarce, with many clinicians adopting the subjective 'eyeball test' for assessing frailty in LT listing candidates (234). Consequently, the prevalence of physical frailty remains unknown in non-American countries and the ability to identify LT candidates most suitable for therapeutic intervention (i.e. nutrition, exercise, psychological support) is therefore limited.

The Liver Frailty Index (LFI), by Lai and colleagues (146), is the most studied tool for physical frailty to date, consisting of three performance-based measures of physical function and strength (hand grip strength, balance and chair stands). LFI is simple, quick (3-5 minutes), can be carried out in any clinical setting (including outpatient clinic) and is reproducible (235). In several centres in North America, the LFI has been shown to predict waiting list mortality, hospitalisation and outcomes post-LT (137, 236, 237). However, it has not been studied or

validated outside of North America. Despite the positive contribution of the LFI to physical frailty assessments, it does not incorporate all aspects of physical frailty i.e. the direct assessment of functional aerobic capacity (functional capacity) (the ability to efficiently use oxygen, e.g. endure physiological stress during a LT). Measures of functional capacity, such as the six-minute walk test (6MWT) and cardiopulmonary exercise testing (CPET) have proved useful in predicting LT waiting list mortality (64, 153, 160). However, 6MWT is limited in accuracy, practicability (i.e. need for a 30-meter level indoor walking course), the volitional nature of the test (i.e. variable level of encouragement by testers) and by patients becoming more familiar with the test (i.e. 'learning effect'). Although more accurate, CPET requires costly equipment, specifically trained staff and can be uncomfortable for patients with CLD, especially those with ascites. Therefore, there is a need for a more accessible and accurate assessment of functional capacity in LT candidates. The Duke Activity Status Index (DASI) is a quick, self-reported 12-item physical activity questionnaire which correlates well with gold-standard assessments of functional capacity (CPET) in patients with chronic cardio-respiratory diseases (163-166). Furthermore, the DASI was able to predict adverse outcomes (30-day mortality, myocardial infarction and one-year new disability) over and above that of CPET and serological tests (i.e. NT Pro-BNP) in 1401 patients undergoing major non-cardiac surgery (238). In view of these results, the ease of assessment and the cost-savings of completion; the investigation of the validity of DASI, alongside the LFI, warrants investigation in LT candidates.

Accurate assessment of a patient's physiological reserve and ability to cope with the physical stressors of LT remain key. Simple tools of physical frailty (e.g. LFI) and functional capacity

(e.g. DASI) exist, but lack wide-spread validation in LT candidates. Therefore, the aim of this prospective, observational study were to:

1. Determine the prevalence, severity and predictors of physical frailty in patients assessed for LT.
2. Investigate the ability of the LFI (physical frailty) and DASI (functional capacity) to predict overall mortality, waiting list mortality, and Intensive Care Unit (ICU) length of stay.

2.2 Methods

2.2.1 Study overview and population

A single centre, prospective observational cohort study was conducted at the LT Unit, Queen Elizabeth University Hospital Birmingham (QEUHB), UK. A service quality improvement audit code (ID: 15209) was obtained from QEUHB clinical governance and ethics department in 2018. Between 1st September 2018 and 1st September 2019 adult patients (≥18years) were consecutively recruited from the LT outpatient assessment and waiting list clinics at QEUHB. Patients were excluded if they were unable to consent or unable to complete one or more of the tests - due to urgent hospital admission for acute illness, severe hepatic encephalopathy (grade ≥3 or 4) or an acute musculoskeletal injury impeding completion of one or more elements of the tests. On average between 250 and 350 participants are listed for a liver transplant at QEUHB per year, with an estimated 10% non-transplant related mortality. Therefore, we aimed to recruit 300 patients over the recruitment period.

2.2.2 Study procedures

In addition to the routine out-patient clinic visit procedures, study participants were asked to complete the DASI questionnaire and the LFI under the supervision of trained personnel. Participants and clinicians were *blinded* to the results of the DASI and LFI in order to avoid study bias and any potential influence on organ allocation and/or LT waiting list status. Assistance in the form of reading the DASI questions and circling the answer, from either study personnel or the caregiver/translator, was given for those who were unable to independently complete the questionnaire (i.e. those with grade 1-2 hepatic encephalopathy or English not their first language). Study personnel, patients and caregivers were encouraged to ensure that the answers were provided by the patient alone. The self-reported DASI questionnaire consists of 12 questions related to functional capacity (i.e. can you climb a flight of stairs?) and is scored from 0 to 58.2, with the latter representing the highest functional status. The DASI score was converted into estimated VO_2 peak using the following equation: VO_2 peak (mL/kg) = 0.43 x DASI + 9.6 (238). Physical frailty was measured using the LFI (146), whereby every patient was asked to complete the following three performance-based measures:

1. *Hand grip strength*: The participant was asked to stand up straight with their dominant arm straight down by their side holding the hand dynamometer (Takei, 5401 GRIP-D). The participant was instructed to squeeze the dynamometer as hard as they could for five seconds. This was repeated three times, with a one minute rest between each test.
2. *Timed 5 x chair stands*: Using the same chair and with the patient folding their arms across their chest, the number of seconds required to complete 5 chair stands was recorded.

3. *Balance testing*: The participant was asked to adopt three balance positions (feet together, semi tandem and tandem) and the time that each three positions were held was recorded, up to a maximum of 10 seconds for each position.

The results of each test were inputted into the online LFI calculator available at http://liverfrailtyindex.ucsf.edu, where a continuous score was provided and the patient categorised as robust (score = <3.2), pre-frail (score = 3.2-4.5) or frail (score = >4.5). The LFI scores for all participants were plotted against the scores provided by the US cohort (146) for comparison of levels of physical frailty between the continents.

2.2.3 Data collection

Demographic data were collected from the patient's electronic health records and laboratory blood sampling (full blood count, urea and electrolytes, liver function tests, international normalized ratio [INR]) on the same day of their clinic visit and completion of the LFI and DASI. Disease aetiology, severity (Model for End-stage Liver Disease [MELD], United Kingdom Model for End-stage Liver Disease [UKELD], history of variceal bleed, hepatic encephalopathy, and ascites) and key medical co-morbidities (i.e. ischaemic heart disease, atrial fibrillation, type 2 diabetes, hypertension, smoking history) were recorded. Body mass index (BMI) was calculated based on the participants estimated dry total body weight, which was corrected for the presence of ascites and peripheral oedema (239). Participants were prospectively followed up until the censor date of the study of 31st of May 2020, with regards to overall mortality, waiting list mortality, and post-LT ICU length.

2.2.4 Statistical analysis

Data was analysed using IBM SPSS statistics software (version 28.0) and R statistics (version 4.1.2). Participant demographics were presented as mean (SD), median (IQR) and number (percentages) depending on the variable. Level of significance was set at $p<0.05$ for all statistical tests.

Single and multiple regression analysis were ran between LFI and DASI with other patient variables (UKELD, MELD, age, sex, BMI, diabetes, variceal bleed, ascites, hepatic encephalopathy, sodium, creatinine, bilirubin, INR, white blood cells, and neutrophil-to-lymphocyte ratio) was completed. Note single regression analysis was only used for the variables UKELD and MELD due to a correlation of 0.8 between those variables and the involvement of variables that composite these measures already in the regression model. Due to skewness of the DASI for the regression analysis the log of DASI (+ 1 to account for 0 scores) was used for the outcome variable. Regression analysis was also used to compare LFI (and its individual components) between patients with and without cirrhosis and sex (male versus female). Of note, balance (one component of LFI) was excluded from the comparison of the above groups due to minimum variability in that measure (89% had a perfect score of 30).

Waiting list mortality was defined as the outcome of 'death' whilst on the waiting list. Follow-up time for those who did not die or receive a LT was censored on 31st May 2020. Survival analysis (overall and waiting list) for those listed for LT was calculated using Cox Survival analysis for both LFI and DASI. Kaplan Meier curves for the Cox Survival models were looked at for any proportional hazards assumption violations. ICU length of stay was defined as the

time (days) from admission to ICU to the time of discharge to the ward for those who underwent a LT. Cox Survival analysis was used to calculate the relationship between LFI and ICU length of stay, as well as DASI and ICU length of stay. There was no need to adjust for competing risks in this model as there were no deaths during an ICU stay.

2.3 Results

2.3.1 Patient demographics

A total of 307 patients were recruited from the LT assessment and waiting list clinic at QEUHB, over a 12-month period from 1st September 2018. 57% (175/307) participants were male, median age was 54 (inter quartile range (IQR) 45-61) years and median dry BMI was 27.8 kg/m^2 (IQR 24-33). The median UKELD score was 52 (IQR 49-55) and the most prevalent ESLD disease aetiology was alcohol-related liver disease (ArLD) at 34% (103/307). Decompensated liver disease was the main indication for LT assessment in 78% (238/307), with the remaining 23% assessed for recurrent cholangitis (n=21), polycystic liver disease (n=14), hepatocellular carcinoma (n=9), LT graft failure (n=5) and other (n=15) (i.e. non-cirrhotic portal hypertension, cystic fibrosis, glycogen storage disease). 38% (117/307) of all participants had grade I-II (west-haven (100)) hepatic encephalopathy and 42% (129/307) had ascites requiring diuretics and/or abdominal paracentesis. The most common medical comorbidities included central obesity (37%), hypertension (26%) and type 2 diabetes (25%), with 27% (82/307) of the cohort having two or more metabolic risk factors (**Table 2.1**).

Table 2.1 Characteristics of study cohort overall and by level of physical frailty (based on LFI)

Characteristic	Overall (n=307)	Frail (n=47)	Pre-frail (n=201)	Robust (n=59)
Sex (male)	175 (57%)	26 (55%)	107 (53%)	42 (71%)
Age (years)	54 (45, 61)	57 (52, 61)	55 (45, 62)	48 (36, 56)
BMI kg/m^2	27.8 (24, 33)	31 (26, 34)	28 (24, 34)	26 (24, 30)
BMI > 30 kg/m^2	114 (37%)	24 (51%)	74 (37%)	16 (27%)
Aetiology:				
ArLD	103 (34%)	25 (53%)	67 (33%)	11 (19%)
NAFLD	52 (17%)	10 (21%)	31 (15%)	11 (19%)
Immune (AIH, PSC, PBC)	69 (22%)	7 (15%)	45 (22%)	17 (29%)
HCC	19 (6%)	0 (0%)	13 (7%)	6 (10%)
Other	64 (21%)	5 (11%)	45 (22%)	14 (24%)
Severity of Liver disease:				
Cirrhosis	239 (78%)	42 (89%)	160 (80%)	37 (63%)
UKELD	52 (49, 55)	52 (50, 55)	52 (49, 55)	51 (47, 54)
MELD	13 (9, 16)	11 (10, 16)	13 (9, 16)	13 (9, 15)
Variceal haemorrhage	48 (16%)	6 (13%)	33 (16%)	9 (15%)
Hepatic Encephalopathy	117 (38%)	26 (55%)	77 (38%)	14 (24%)
Ascites	129 (42%)	27 (57%)	90 (48%)	12 (20%)
Comorbidities:				
Hypertension	79 (26%)	9 (19%)	61 (30%)	9 (15%)
Type 2 Diabetes	78 (25%)	11 (23%)	57 (28%)	11 (19%)
Atrial Fibrillation	8 (3%)	2 (4%)	6 (3%)	0 (0%)
Ischaemic Heart Disease	4 (1%)	1 (2%)	3 (2%)	0 (0%)
≥2 metabolic components	82 (27%)	11 (23%)	63 (31%)	8 (14%)
Smoking history:				
Non-smoker	184 (60%)	26 (55%)	117 (58%)	41 (69%)
Ex-smoker	107 (35%)	18 (38%)	74 (37%)	15 (25%)
Current smoker	16 (5%)	3 (6%)	10 (5%)	3 (5%)
Physical frailty:				
LFI*	3.82 (0.72)	4.95 (0.39)	3.85 (0.36)	2.83 (0.37)
DASI (scale 0-58)**	28.7 (16.2, 50.2)	15 (10, 21)	29 (18, 43)	51 (38, 58)
Outcomes:				
Overall Death	38 (12%)	7 (15%)	29 (14%)	2 (3%)
Underwent LT	159 (52%)	15 (32%)	111 (55%)	33 (56%)
ICU length of stay (days)	2.0 (1.0, 5.0)	3.0 (2.5, 5.0)	2.0 (2.0, 5.0)	2.0 (1.0, 5.0)
Hospital length of stay (days)	12 (9, 18)	9 (8, 16)	12 (9, 18)	11 (9, 19)

Note: Data expressed as n (%), mean (standard deviation) or median (interquartile range)
*high = more frail; **low = worse functional capacity
Abbreviations: AIH = autoimmune hepatitis, ArLD = alcohol related liver disease, BMI = body mass index, DASI = duke activity status index, HCC = hepatocellular carcinoma, ICU = intensive care unit, LFI = liver frailty index, LT = liver transplantation, MELD = model for end-stage liver disease, NAFLD = non-alcohol related liver disease, PBC = primary biliary cholangitis, PSC = primary sclerosing cholangitis

2.3.2 Patient outcomes after LT assessment

Of the 307 participants who underwent LT assessment, 255 (83.1%) were placed on the LT waiting list and 52 (16.9%) were not listed. The median length of study follow-up from recruitment was 460 (IQR 325-551) days. Reasons for not being listed for LT included too high risk (i.e. frail, cardiac, risk of alcohol relapse) (75%), no active/current LT indication (19%) and progression of HCC outside of criteria (6%). Of those listed for LT, 159/255 (62.4%) underwent LT in the study time-frame, whilst 80/255 (31.4%) were alive and still on the LT waiting list (**Figure 2.1**). Sixteen (6.3%) patients died whilst waiting for a LT, with cause of deaths being primarily liver-related (n=6) and non-liver related (n=10, including COVID-19, infection/sepsis, multi-organ failure). Median length of follow-up post LT was 354 (IQR 247-453) days, with a median length of stay on ICU and in hospital post LT was 2 (IQR 1-5) and 12 (IQR 9-18) days, respectively. There was a total of 7 (4.4%) deaths post-LT, two within 30 days (1x intraoperative haemorrhage, 1x sepsis and multi-organ failure), one within 90 days (cause unknown), and four post-90 days (two due to COVID-19, one advancing neuroendocrine tumour, and one metastatic recurrence of HCC). 30-day and 90-day post-LT mortality was 1.3% (2/159) and 0.6% (1/159), respectively.

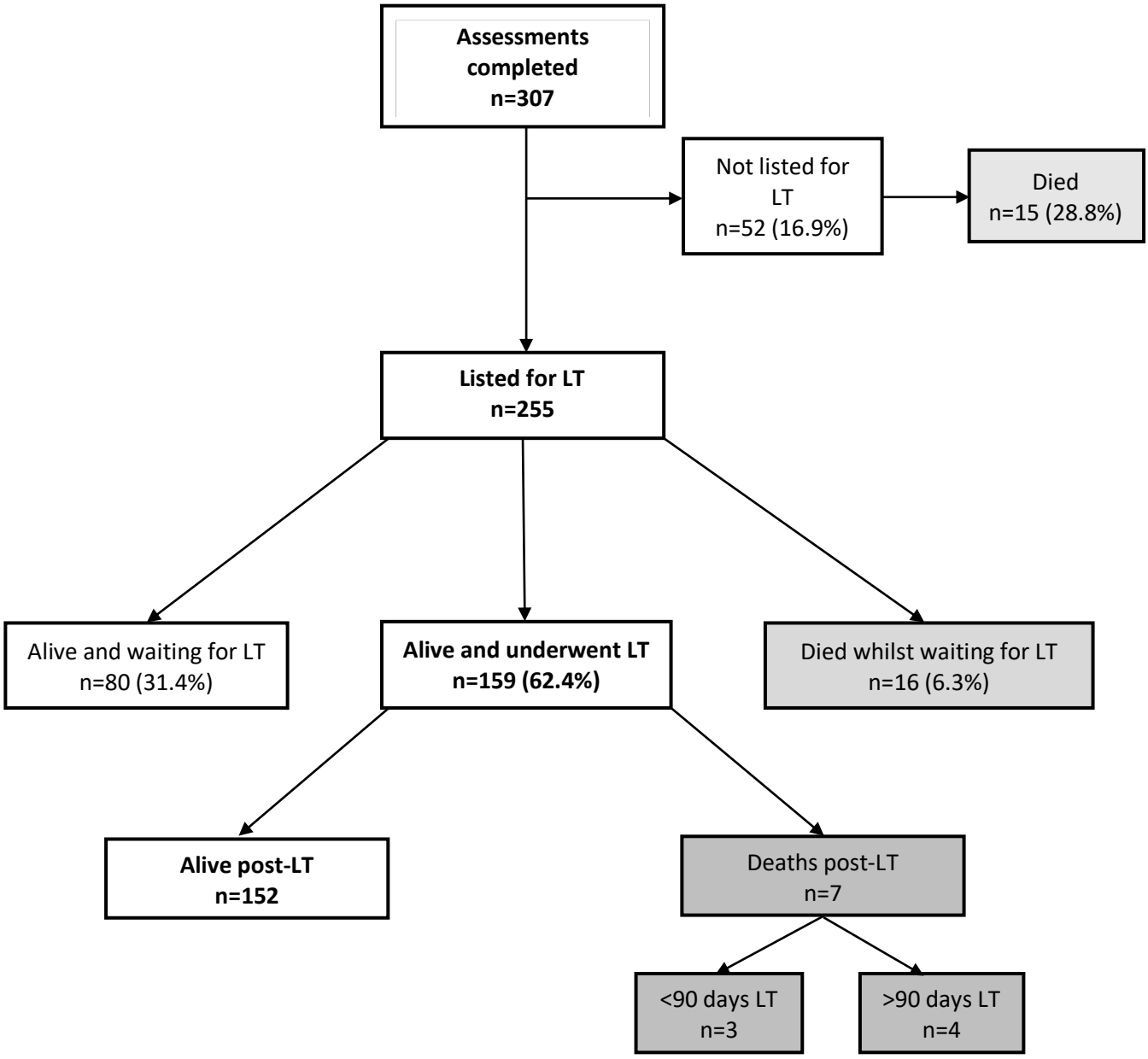


Figure 2.1 Flow diagram of patient journey

2.3.3 Aim 1: Prevalence and predictors of physical frailty (LFI)

At study baseline, the mean LFI score was 3.82 (SD=0.72), with 19% (59/307) classified as robust, 65% (201/307) pre-frail and 15% (47/307) frail, which was similar to that presented by the United States (US) group in 2017 (146) (**Figure 2.2**). Single regression analysis showed that age (regression coefficient, B=0.012, 95% CI 0.006-0.018, p<0.001), hepatic encephalopathy

(B=0.386, 95% CI 0.224 to 0.548, p<0.001), ascites (B=0.274, 95% CI 0.112 to 0.435, p=0.001), sodium (B=-0.047, 95% CI -0.066 to -0.027, p<0.001), INR (B=0.306, 95% CI 0.069 to 0.543, p=0.012), UKELD (B=0.023, 95% CI 0.005-0.04, p=0.01) and the presence of cirrhosis (B=0.279, 95% CI 0.086 to 0.473, p=0.005) were all significantly associated with a higher LFI (i.e. increased physical frailty). In multiple regression analysis, age (B=0.009, 95% CI 0.002 to 0.015, p=0.008), female sex (B=0.275, 95% CI 0.114 to 0.437, p=0.001), hepatic encephalopathy (B=0.275, 95% CI 0.094 to 0.456, p=0.003), and sodium (B=-0.041, 95% CI -0.063 to -0.02, p<0.001) were the strongest independent predictors of high LFI (adjusted R^2=0.15) (**Table 2.2**). Additionally, individual components of the LFI (chair stands and hand grip strength) were analysed against variables of interest including sex and the presence or absence of cirrhosis at LT assessment. Females and those with cirrhosis were significantly slower at performing five chair stands than males and those without cirrhosis (0.38 vs. 0.43 chair stands per second (cs/sec), p=0.046 and 0.39 vs. 0.47 cs/sec, p=0.005), respectively. Females had significant lower hand grip strength than males (20.8 vs. 34.2 kg, p<0.001), but the presence or absence of cirrhosis did not impact upon hand grip strength (28.48 vs. 28.20kg, p=0.83). Analysis for balance was not reasonable as 89% of all participants scored the maximal 10/10.

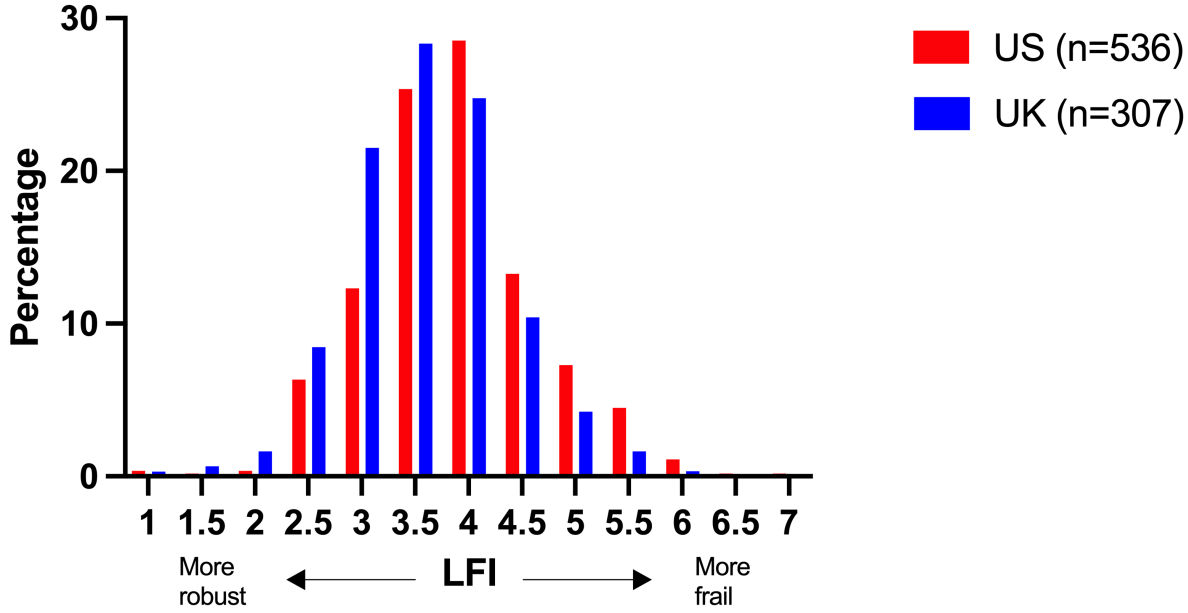


Figure 2.2 Comparison of LFI scores from a UK and United States (US) dataset
Note: US dataset taken from Lai et al (2017) (146)

Table 2.2 Unadjusted and adjusted regressions of LFI in patients assessed for a LT

Variable	Univariate Coefficient (95% CI)	P-value	Multivariate Coefficient (95% CI)	P-value
Age	0.012 (0.006-0.018)	<0.001*	0.009 (0.002-0.015)	0.008*
Female sex	0.154 (-0.01-0.318)	0.065	0.275 (0.114-0.437)	0.001*
Dry BMI	0.01 (-0.003-0.023)	0.116	0.001 (-0.012-0.014)	0.871
UKELD	0.023 (0.005-0.04)	0.01*	-	-
MELD	0.008 (-0.01-0.027)	0.373	-	-
Cirrhosis	0.279 (0.086-0.473)	0.005*	0 (-0.224-0.224)	1.00
Ascites	0.274 (-0.112-0.435)	0.001*	0.038 (-0.144-0.219),	0.683
Hepatic encephalopathy	0.386 (0.224-0.548)	<0.001*	0.275 (0.094-0.456)	0.003*
Diabetes	0.128 (-0.06-0.317)	0.188	-0.011 (-0.197-0.175)	0.907
Significant varices	0.025 (-0.197-0.248)	0.823	-0.047 (-0.261-0.167)	0.667
Sodium	-0.047 (-0.066- -0.027)	<0.001*	-0.041 (-0.063- -0.02)	<0.001*
Creatinine	0 (-0.001-0.001)	0.359	0 (-0.001-0.001)	0.664
Bilirubin	0 (-0.001-0.001)	0.89	-0.001 (-0.003-0)	0.077
INR	0.306 (0.069-0.543)	0.012*	0.221 (-0.033-0.475)	0.088
WBC	0.013 (-0.021-0.047)	0.456	0.011 (-0.024-0.046)	0.528
NLR	0.005 (-0.013-0.023)	0.578	-0.001 (-0.019-0.016)	0.865

Note: Due to the inclusion of identical variables, MELD and UKELD were not included in the multivariate analysis.
*significant variable.
Abbreviations: BMI = body mass index, CI = confidence interval, INR = international normalised ration, LFI = liver frailty index, MELD = model for end-stage liver disease, NLR = neutrophil to lymphocyte ratio, UKELD = United Kingdom model for end-stage liver disease, WBC = white blood cell count.

2.3.4 Aim 1: Prevalence and predictors of poor functional 'exercise' capacity (DASI)

At study baseline, the median DASI score and estimated VO_2 peak were 28.7 (IQR 16.2 to 50.2) and 21.9ml/kg/min (IQR 16.6 to 31.2) respectively. The DASI significantly correlated with LFI (r=-0.62, p<0.001), in that the lower the DASI (lower functional capacity) the higher the LFI (more frail) (**Figure 2.3**). In single regression analysis, female sex (B=0.811, 95% CI 0.694 to 0.946, p=0.008), dry BMI (B= 0.984, 95% CI 0.972 to 0.996, p=0.008), ascites (B=0.807, 95% CI 0.690 to 0.943, p=0.007), hepatic encephalopathy (B=0.834, 95% CI 0.710 to 0.979, p=0.027) and sodium (B=1.028, 95% CI 1.009 to 1.047, p=0.004) were significantly associated with lower DASI scores (lower functional capacity). However, in multiple regression analysis only female sex (B=0.739, 95% CI 0.63 to 0.868, p<0.001), BMI (B=0.981, 95% CI 0.969 to 0.995, p=0.006) and sodium (B=1.025, 95% CI 1.005 to 1.047, p=0.017) were independent predictors of low DASI scores (adjusted R^2=0.09) (**Table 2.3**).

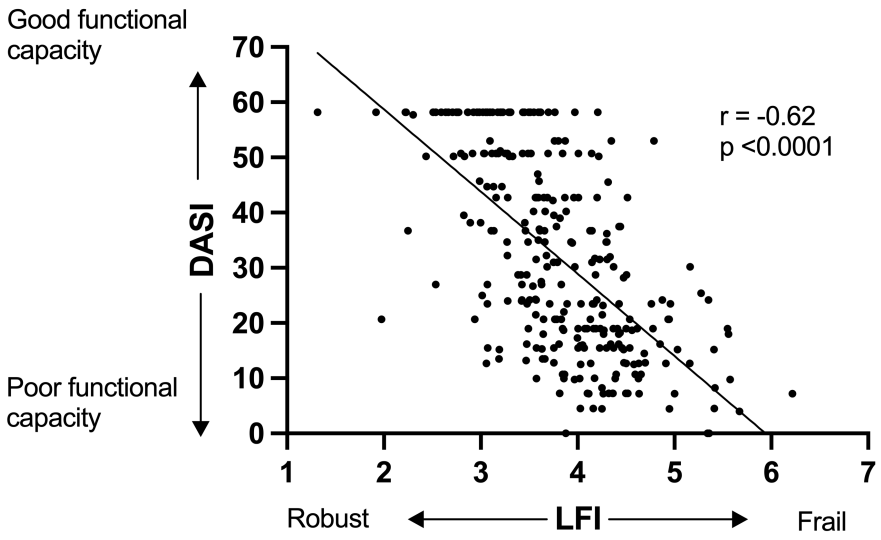


Figure 2.3 Correlation between Liver Frailty Index (LFI) and Duke Activity Status Index (DASI) Note: As frailty (LFI) increases, functional capacity (DASI) declines.

Table 2.3 Unadjusted and adjusted regression of DASI in patients assessed for a LT

Variable	Univariate Coefficient (95% CI)	P-value	Multivariate Coefficient (95% CI)	P-value
Age	0.996 (0.99-1.002)	0.185	1 (0.993-1.006)	0.945
Female sex	0.811 (0.694-0.946)	0.008*	0.739 (0.63-0.868)	<0.001*
BMI	0.984 (0.972-0.996)	0.008*	0.981 (0.969-0.995)	0.006*
UKELD	0.988 (0.972-1.005)	0.165	-	-
MELD	0.995 (0.977-1.012)	0.556	-	-
Cirrhosis	0.888 (0.735-1.012)	0.212	1.025 (0.822-1.279)	0.824
Ascites	0.807 (0.69-0.943)	0.007*	0.879 (0.735-1.052)	0.159
Heptaic encephalopathy	0.834 (0.71-0.979)	0.027*	0.942 (0.787-1.125)	0.507
Diabetes	0.944 (0.787-1.132)	0.531	1.033 (0.857-1.244)	0.732
Significant varices	0.906 (0.732-1.12)	0.357	0.891 (0.722-1.102)	0.288
Sodium	1.028 (1.009-1.047)	0.004*	1.025 (1.005-1.047)	0.017*
Creatinine	1 (0.999-1.001)	0.903	1 (0.999-1.001)	0.711
Bilirubin	1 (0.998-1.001)	0.583	1.001 (0.999-1.002)	0.256
INR	0.874 (0.689-1.108)	0.266	0.943 (0.734-1.23)	0.662
WBC	0.968 (0.936-1)	0.05	0.967 (0.933-1.002)	0.063
NLR	0.988 (0.972-1.005)	0.166	0.997 (0.98-1.014)	0.721

Note: Due to the inclusion of identical variables, MELD and UKELD were not included in the multivariate analysis. Also, since DASI was log transformed and the coefficients above have been transformed back these represent a percent increase rather than a point increase.
*significant variable
Abbreviations: BMI = body mass index, DASI = duke activity status index, INR = international normalised ration, MELD = model for end-stage liver disease, NLR = neutrophil to lymphocyte ratio, UKELD = United Kingdom model for end-stage liver disease, WBC = white blood cell count

2.3.5 Aim 2: Predictors of overall and waiting list mortality

The overall mortality for the study population was 12.4% (38/307). Both LFI (HR=2.04, 95% CI 1.31 to 3.16, p=0.001) and DASI (HR=0.97, 95% CI 0.95 to 0.99, p=0.001) were significantly related to overall mortality (concordance=0.64 [LFI] and 0.68 (DASI)) (**Figure 2.4A**). When UKELD is added to the models, both LFI (HR=1.94, 95%CI 1.24 to 3.03, p=0.004) and DASI (HR=0.97, 95%CI 0.95 to 0.99, p=0.002) remained significant predictors of all-cause mortality, with marginal improvements in concordance (0.70 for LFI+UKELD and 0.73 for DASI+UKELD) (**Table 2.4** and **Table 2.5**).

6.4% (16/255) of participants died on the LT waiting list. Again, both LFI (HR=1.94, 95% CI 1.03 to 3.68, p=0.042) and DASI (HR=0.96, 95% CI 0.93 to 0.99, p=0.020) predicted LT waiting list mortality (**Table 2.4** and **Table 2.5**, **Figure 2.5).** When the UKELD was added to the models the LFI (HR=1.68, 95% CI 0.93 to 3.05, p=0.088) was insignificant while the DASI (HR=0.96, 95% CI 0.93 to 1.00, p=0.037) remained significant, despite increased concordance to 0.80 and 0.85, respectively.

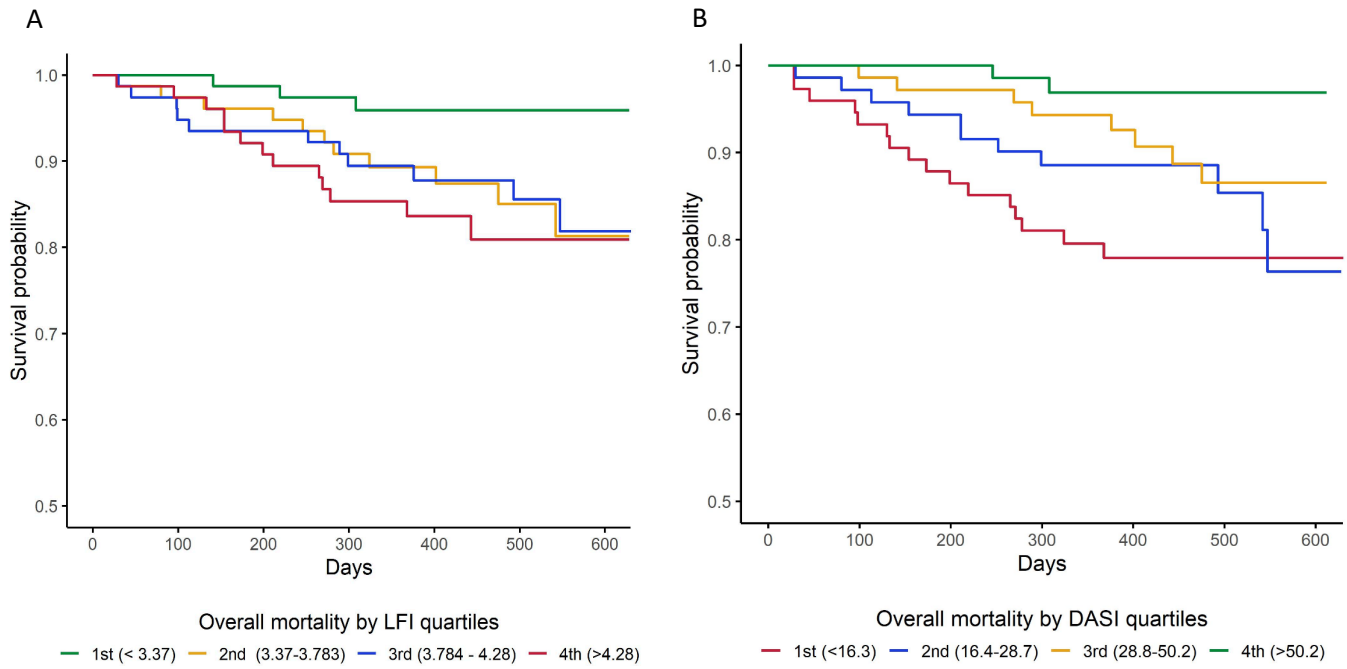


Figure 2.4 Overall mortality by (A) LFI quartiles and (B) DASI quartiles
Note: A high LFI score relates to increased frailty, whereas a high DASI score indicates higher functional capacity.

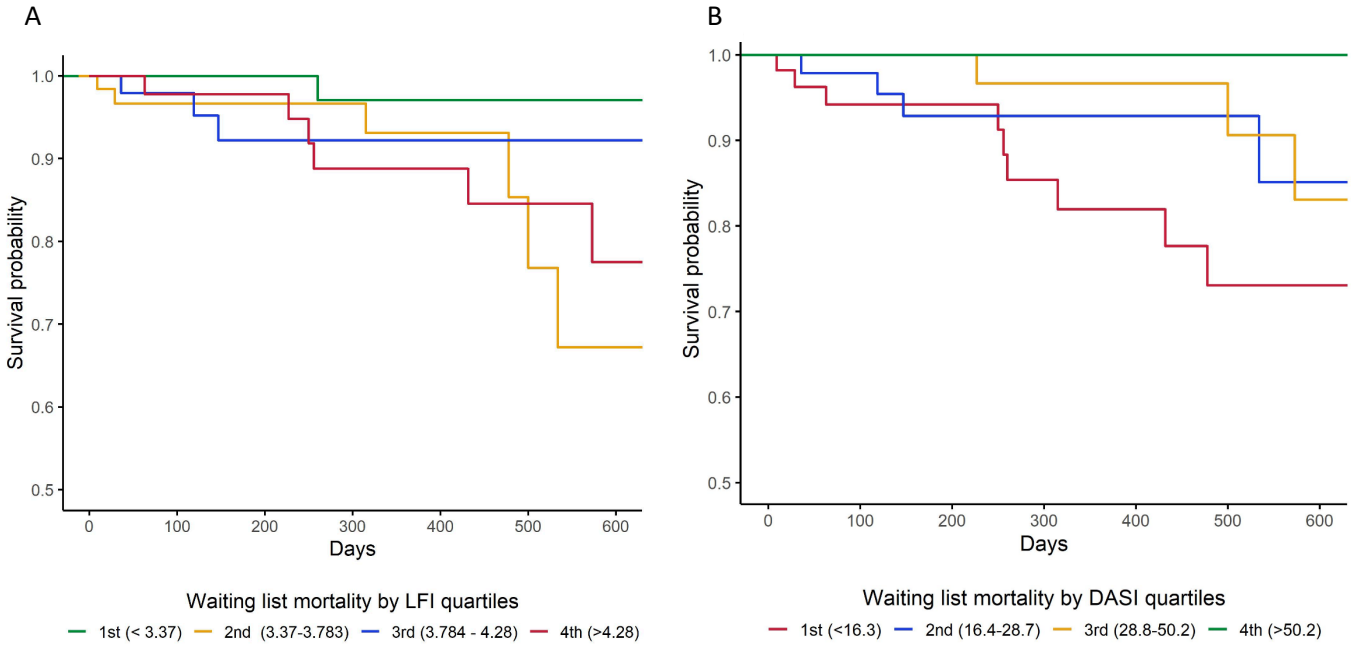


Figure 2.5 Waiting list mortality by (A) LFI quartiles and (B) DASI quartiles
Note: A high LFI score relates to increased frailty, whereas a high DASI score indicates higher functional capacity.

Table 2.4 Overall mortality, waiting list mortality and ICU length of stay Cox survival models with LFI

Variable	Overall mortality			Waiting list mortality			ICU length of stay		
	HR (95% CI)	P-value	Concordance	HR (95% CI)	P-value	Concordance	HR (95% CI)	P-value	Concordance
LFI	2.04(1.31-3.16)	0.001	0.64	1.94 (1.03-3.68)	0.042	0.63	0.84 (0.66-1.07)	0.157	0.59
UKELD	1.11 (1.05-1.19)	0.001	-	1.29 (1.16-1.44)	<0.001	-	0.99 (0.96-1.03)	0.71	-
LFI+UKELD	1.94 (1.24-3.03)	0.004	0.70	1.68 (0.93-3.05)	0.088	0.80	0.85 (0.66-1.09)	0.193	0.60

Abbreviations: CI = confidence interval, HR = hazard ratio, ICU = intensive care unit, LFI = liver frailty index, UKELD = United Kingdom model for end-stage

Table 2.5 Overall mortality, waiting list mortality and ICU length of stay Cox Survival Models with DASI

Variable	Overall mortality			Waiting list mortality			ICU length of stay		
	HR (95% CI)	P-value	Concordance	HR (95% CI)	P-value	Concordance	HR (95% CI)	P-value	Concordance
DASI	0.97 (0.95-0.99)	0.001	0.68	0.96 (0.93-0.99)	0.020	0.73	1.00 (1.00-1.01)	0.405	0.56
UKELD	1.11 (1.04-1.17)	0.001	-	1.28 (1.15-1.41)	<0.001	-	0.99 (0.96-1.02)	0.554	-
DASI+UKELD	0.97 (0.95-0.99)	0.002	0.73	0.96 (0.93-1.00)	0.037	0.85	1.00 (0.99-1.01)	0.446	0.59

Abbreviations: CI = confidence interval, DASI = duke activity status index, HR = hazard ratio, ICU = intensive care unit, UKELD = United Kingdom model for end-stage liver disease

2.3.6 Aim 2: Predictors of ICU length of stay

Neither LFI (HR=0.84, 95% CI 0.66 to 1.07, p=0.157), DASI (HR=1.00, 95% CI 1.00 to 1.01, p=0.405) nor when UKELD was added (HR 0.85 [LFI] and 1.00 [DASI], p>0.5 in both models) were significantly related to ICU length of stay (**Table 2.4** and **Table 2.5**).

2.4 Discussion

Physical frailty and functional capacity are important components of risk assessment for all types of major surgery. Healthcare practitioners' subjective ['eyeball test'] assessment of patients' frailty and functional capacity has uncertain accuracy and does not enable therapeutic targets pre-operatively. Our prospective, single-centre UK study highlights that both physical frailty and poor functional capacity, as determined by simple easy-to use tools (LFI, DASI), are common in patients assessed for and undergoing LT, with only 19% of patients defined as "robust". Furthermore, both physical frailty and poor functional capacity measured at the time of LT assessment predicted waiting list and overall mortality. Both female sex and hyponatraemia were independent predictors of both physical frailty (high LFI) and poor functional capacity (low DASI). In addition, older age and hepatic encephalopathy predicted physical frailty, whilst high BMI predicted poor functional capacity. Understanding and identifying those patient groups that are higher risk of physical frailty, poor functional capacity and subsequent mortality at the point of LT assessment, may aid with targeting future prehabilitation programmes (nutrition, exercise, psychology).

In out-patient liver departments, in which time and space can be limited, evaluation of functional capacity has remained a challenge in patients with ESLD. Our study is the first to investigate the utility of the DASI questionnaire in this LT setting. Not only is the DASI questionnaire user-friendly, cost-effective, time efficient (<5 minutes), but it provides a simpler alternative to either the 6MWT (requires 30 metre space, healthcare supervision) or CPET (expensive, expert supervision/equipment), in predicting overall and waiting list mortality in patients undergoing a LT. Whilst the DASI questionnaire is limited by its patient subjectivity, it has previously been shown to correlate well with the gold standard measure of CPET, in patients with chronic cardiorespiratory diseases and those undergoing non-cardiac surgery (163-166). Similar to our findings in patients awaiting LT, Wijeysundera and colleagues (238), highlighted in 1401 patients undergoing major non-cardiac surgery (NB not LT) that the DASI was able to predict 30-day and 1-year survival. Similarly, Ney and colleagues performed a meta-analysis of CPET in 1107 patients and highlighted that functional capacity (i.e. weighted mean VO_2 peak) was below the threshold required for independent living in ESLD and was associated with pre-and post-LT survival (64). Despite these significant findings, the use of CPET in the LT setting is not uniform throughout Europe and the US, largely as a result of cost, specialist equipment, workforce requirement and perception that the logistical burden of CPET outweighs the additional information provided to guide patient care (234). Based on our findings, even in those LT centres with the expertise and facilities for CPET, the DASI may be utilised as a quick, cheap screening tool in out-patients to determine who may need or may not need more intricate analysis and individualised prehabilitation.

The prevalence of physical frailty within our UK-based study and its ability to predict mortality is similar to that reported by Lai and colleagues in the US (**Figure 2.2**) (146, 149); thereby further validating the use of LFI in patients being assessed for a LT. Most notably, in our study, female sex was a predictor of both physical frailty and poor functional capacity. In particular, females performed significantly worse on the hand grip strength and chair stand components of the LFI. This finding is supported by a multicentre cohort US study (2020) of 1405 patients with cirrhosis waiting for LT, in which females presented with worse physical frailty scores despite similar liver disease severity. Moreover in the US study, physical frailty accounted for 13% of the known gender gap in waiting list mortality (240). Socioeconomic status and/or sociocultural experiences may contribute to the gender variations seen in physical frailty, in addition to the more widely recognised physical differences, such as biological or genetic factors (241). These findings are important, because unlike factors such as liver disease severity and age, physical frailty is a potentially modifiable contributor of waiting list mortality (183). Future studies should focus on gender-specific preventative and restorative programs for physical frailty in ESLD.

In addition to female sex and age, key clinical determinants of the severity of liver failure (including hyponatraemia, hepatic encephalopathy, ascites and UKELD) were all significant predictors of increased physical frailty in our cohort. In addition, patients with cirrhosis performed significantly worse in the physical frailty subscale, chair stands, than those with non-cirrhotic disease aetiologies, such as recurrent cholangitis (e.g. PSC) and polycystic liver disease. These findings may be explained by the mechanisms driving physical frailty in cirrhosis (i.e. chronic inflammation, 'accelerated starvation' state/malnutrition, hyperammonaemia)

(197), which ultimately result in disruption of the maintenance of muscle health. For example the chronic inflammatory state seen in cirrhosis is associated with an increase in myostatin, which itself inhibits muscle protein synthesis, satellite cell activity and increases proteolysis (242); thereby resulting in skeletal muscle loss and associated reduced survival (81). Furthermore, increases in circulating pro-inflammatory cytokines, such as IL-6 and TNF-α, contribute to increased muscle catabolism (243, 244).

Hepatic encephalopathy and ascites are the two most common debilitating complications of ESLD (245, 246), with both being strongly associated with physical frailty and reduced functional capacity in our study. Due to reduced hepatic function and/or portal systemic shunting those with hepatic encephalopathy have higher levels of circulating ammonia (247), which directly upregulates myostatin and subsequently impacts on muscle protein, *as described above* (248, 249). Furthermore, hyperammonemia increases mitochondrial dysfunction (250), thus it is not surprising that we found hepatic encephalopathy to be associated with reduced functional capacity. Patients with ascites, as highlighted by our study, are particular susceptible to physical frailty likely due to reduced appetite, early satiety, delayed gut motility (251), and subsequent decreased calorie intake; all of which exacerbate the state of 'accelerated starvation' (impaired hepatic glycogen stores) found in cirrhosis (197). Additionally, due to the associated weight burden and shortness of breath experienced by those with ascites, physical inactivity is more prevalent, further perpetuating skeletal muscle loss (252). Both hepatic encephalopathy and ascites should therefore be optimised (i.e. medications, easy-to-access paracentesis), in parallel to prehabilitation programmes (nutrition/exercise), in order to minimise physical frailty and functional decline prior to LT.

2.4.1 Limitations

There are several limitations with this study. Firstly, patients were recruited from both the assessment and waiting list clinic which meant that patient physical frailty was assessed at varying stages of the patient waiting list period which may have influenced waiting list mortality. In addition, only those who died whilst on the waiting list were included within the waiting list mortality outcome and therefore this did not take into account those who may have been de-listed during the course of the study. However, despite these exclusions, LFI remained significant in predicting waiting list mortality.

Secondly, throughout the study, patients received varying levels of therapeutic intervention, primarily due to limited-service provisions. As such, those who were frail were more likely to have been subjectively selected and prioritised for intervention, which again may have altered the waiting list mortality outcomes. While the LFI measure was blinded from decisions makers, if LFI was related to those who were perceived to be frail and those patients in turn received additional interventions prior to assessment then this would have weakened the relationship between LFI and mortality in our data. Subsequently, despite its significance, the relationship could potentially have been stronger.

Finally, despite the prevalence of physical frailty being similar to that reported in the US, it is important to note that this was a single-centre study which may not be representative of all UK/European LT centres. Therefore, further multicentre European studies are needed to improve the external validity of the results. This should include prospective assessment of LFI and DASI at the time of LT assessment to more accurately evaluate their prediction of waiting

list mortality. Additionally, the impact of interventions, such as exercise and nutrition, on these measures should be investigated.

2.4.2 Contribution to the field

Our study has several strengths. Primarily, this is the first European study to investigate the prevalence and predictive ability of physical frailty (LFI) on overall and waiting list mortality in patients assessed for a LT. The addition of the DASI, which is unique to this study cohort, provides clinicians with a time and cost effective alternative to CPET to identify those most at risk and/or potentially require further in depth investigation of their functional status. The findings highlight the pressing need for other UK/European centres to validate and consider incorporating these simple and cheap measures within routine clinical practice in LT, to enable early identification of those most at risk and to initiate early interventions to targeted patient populations (i.e. female sex, hyponatraemia, older age etc).

2.5 Conclusion

In conclusion, physical frailty and poor functional capacity are highly prevalent in UK patients assessed for LT. The LFI and DASI can help predict LT waiting list and overall mortality associated with the LT clinical pathway. Female sex and hyponatraemia, in particular, are significant predictors for both physical frailty and poor functional capacity in this setting. Implementation of user-friendly and cost effective measures, such as LFI and DASI, should be considered and validated in LT centres throughout the world in order to target interventions (i.e. prehabilitation) prior to LT.

CHAPTER 3:

THE EVALUATION OF PHYSICAL ACTIVITY AND ITS IMPACT ON PHYSICAL FRAILTY IN END-STAGE LIVER DISEASE: METHODS FOR A PROSEPCTIVE OBSERVATIONAL STUDY

3.1 Introduction

In recent years, there has been an increasing interest in the use of exercise and/or physical activity to treat physical frailty in chronic liver disease (CLD). Yet, heterogeneity between study design and various levels of reported adherence, has limited translation of interventions into clinical practice. Further understanding of habitual physical activity patterns and how they impact on physical frailty is needed.

The research conducted herein was completed as part of the wider sarcopenia theme within the Birmingham Biomedical Research Centre at the University of Birmingham. Within this theme a study, titled "evaluation of the mechanisms of sarcopenia in chronic inflammatory disease: a protocol for a prospective observational cohort study (ESCID)", was conducted. Full details of the protocol of this study can be found at https://pubmed.ncbi.nlm.nih.gov/34895316/ (253). I contributed to the development of this protocol and was a co-author on the publication. The aim of the ESCID study was to determine the prevalence of sarcopenia (defined in the protocol as loss of muscle strength, function and mass) in three inflammatory disease states (ESLD, inflammatory bowel disease [IBD] and inflammatory arthritis [IA]) and explore the molecular and lifestyle factors that contribute to sarcopenia found in these disease states. As part of this study I co-led:

1. the recruitment of participants
2. day-today logistics (i.e. study visit booking, MRI booking, muscle biopsy preparation, patient transport etc.)
3. data collection (including blood sampling/processing, muscle ultrasound, muscle biopsy)

4. adverse event reporting and
5. data management.

Furthermore, I analysed data beyond the scope of this thesis including health-related quality of life and sleep. However, for the purpose of this thesis, focus has been given to the physical activity profiles of patients with ESLD and their relation to physical frailty. Therefore, the information presented within this chapter only details the specific methods conducted for the subsequent results Chapters (4 and 5).

3.2 Study overview

A single centre, longitudinal observational, prospective study was conducted to investigate the role of physical activity on physical frailty in patients with ESLD. As part of the ESCID study, patients with ESLD underwent a series of assessments over four defined time points: baseline (visit 1 [V1]), 2-weeks (visit 2 [V2]), 12-weeks (visit 3 [V3]) and 24-weeks (visit 4 [V4]). Throughout the study participants continued to receive standard of care management as determined by their regular specialty team. It was anticipated that during the course of these visits, participants may undergo a liver transplant (LT) which would likely impact on physical activity data. Therefore, physical activity data were collected from the first visit that had valid usable data (defined in section 3.5.1 and 3.5.2), where a LT had not (*yet*) occurred.

In parallel, an age and sex matched healthy control group were recruited to compare physical activity patterns. Healthy controls attended one visit only, therefore all valid data was used from this visit.

3.3 Ethical and Regulatory Approval

The Health Research Authority and West Midlands Solihull Research Ethics Service Committee Authority (REC reference: 18/WM/0167) approved this study. All participants provided informed written consent.

3.4 Study Design

3.4.1 Participant selection

A target of fifty patients with ESLD were recruited from the LT Unit at the Queen Elizabeth University Hospitals Birmingham Foundation Trust (QEHB). Full details of the inclusion and exclusion criteria can be found in **Table 3.1**. A further sample of eighteen age/sex matched healthy controls were recruited for comparison.

Table 3.1 ESCID eligibility criteria

Cohort	Inclusion criteria	Exclusion criteria
End-stage liver disease	Age ≥18 years	Refusal or lack capacity to give informed consent.
	Meeting criteria of liver cirrhosis including all Child Pugh scores from A-C as per British Association for the Study of the Liver guidance.	Currently enrolled in an interventional trial with active treatment for their chronic disease condition.
	Diagnosis or suspected diagnosis of sarcopenia (including evidence of loss of muscle mass and/or functional decline)	Previously undergone liver transplantation or biliary intervention in the ESLD cohort.
		Underlying or active cancer (including known Hepatocellular Carcinoma).
Healthy controls	Age ≥18 years	Refusal or lack capacity to give informed consent.
	No existing chronic inflammatory condition, cancer or significant pre-morbid disease pathology	Any recent (within the last 12-weeks) acute illness or surgery requiring significant treatment or hospitalisation
	No suspicion or evidence of sarcopenia	Any systemic corticosteroid use or replacement
	No previous transplant	Pregnancy

3.4.2 Recruitment and consent

3.4.2.1 End-stage liver disease

Potential eligible patients (**Table 3.1**) were identified by the research team and approached at either the LT assessment or waiting list outpatient clinics. Patients were invited to join the

study via face-face contact, provided with a patient invitation letter and a participant information sheet (PIS). A further 48 hours was given for the patient to consider the study before a telephone call was made by the research team to determine the patient's wish to participate. Once their willingness to participate was confirmed, patients were invited to attend their baseline visit (V1), where written informed consent was obtained. At each contact (face-to-face, telephone, and baseline visit) the patient and their family/friends were given the opportunity to ask questions regarding the study and were advised that they were free to decline the invitation, withdraw from the study at any point, and that participation would not affect their healthcare. A copy of the signed and dated informed consent form was given to the patient, the original was placed in the case report file as well as uploaded on to the hospital electronic patient record, PICS.

3.4.2.2 Healthy controls

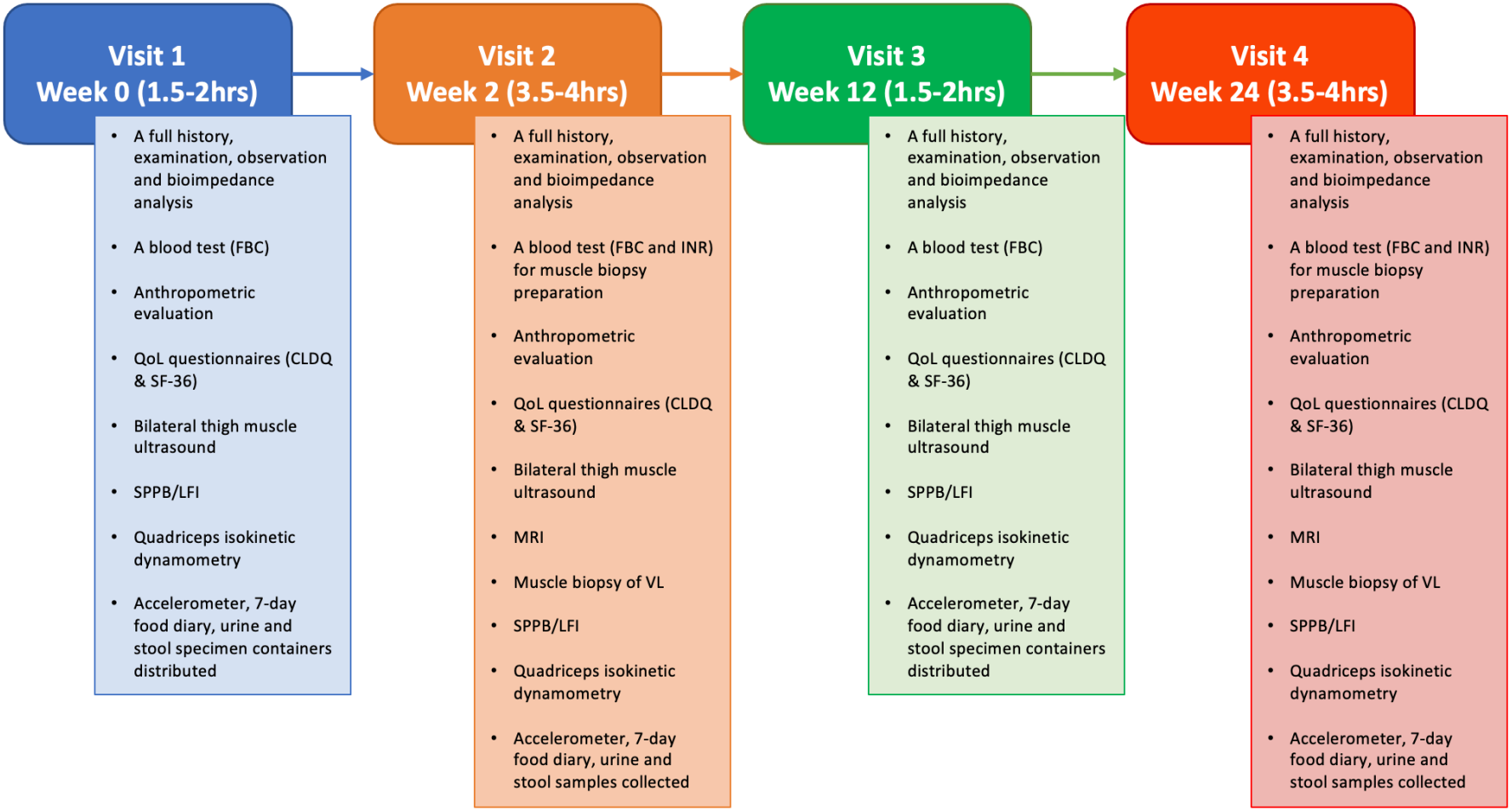
Posters, targeting staff and students, were displayed throughout the University of Birmingham advertising the ESCID study. Individuals who volunteered were screened for eligibility as per **Table 3.1**. Once deemed eligible, volunteers were provided with the PIS to review. A minimum of 48 hours was given before a follow-up call was made by the research team to determine the individual's willingness to participate. Like the ESLD group, participants were given the opportunity to ask questions and advised they were free to withdraw from the study at any point. Written informed consent was obtained at their visit, a copy was given to the participant and the original placed in the case report file as well as uploaded onto the hospital electronic patient record, PICS.

3.5 Study Schedule and Method

All data included in the following results chapters were collated at either V2, V3 or V4 for those with ESLD, and V1, only, for the healthy controls. Data for each visit was collected as described below.

3.5.1 ESLD study visits (V1-V4)

Participants attended either the Inflammatory Research Facility (IRF) or Clinical Research Facility (CRF) at the QEHB on their identified visit day. For those with ESLD, where possible, visits were booked so that they coincided with their regular clinic visits to reduce patient burden. Multiple assessments were completed as part of the ESCID and these are displayed in **Figure 3.1**. Assessments completed specifically in relation to the research studies completed in Chapters 4-5 are shown in **Table 3.2**.



(Figure adapted from Dhaliwal et al 2020)

Figure 3.1 Outline of ESCID study visits, including assessments completed at each visit

Abbreviations: CLDQ = chronic liver disease questionnaire, FBC = full blood count, INR = International Normalised Ratio, LFI = liver frailty index, SF-36 = short-form-36, SPPB = short physical performance battery, VL = vastus lateralis

Table 3.2 Visit assessments

Assessment category	Clinical parameters/outcomes
Past Medical History	Primary ESLD**,** ascites, hepatic encephalopathy, main portal vein thrombosis, portal hypertension**,** DM, HTN; AF, IHD, CVD, CKD, pulmonary disease; musculoskeletal disease, mental health illness
Current medical history/clinical examination	Ascites, SBP, variceal haemorrhage, hepatorenal syndrome/AKI, sepsis, severe hepatic encephalopathy (admission to hospital), chest pain, shortness of breath, palpitations, dizziness/collapse, significant fall, hospital admission, change to medication
Blood samples	FBC, U&E, LFTs, INR, ammonia
Social History	Smoking status, alcohol intake
Anthropometry	Height (cm), estimated dry weight (kg) (using the 5/10/15% reduction rule for mild/moderate/severe ascites; 5% for peripheral oedema), estimated dry BMI (kg/m2)
Physical activity	Accelerometer – outcome measures described in detail in sections 3.5.1 and 3.5.2.
Physical function/strength	LFI, isokinetic dynamometry (lower limb and upper limb), gait speed,
Muscle mass	Thigh muscle ultrasound (VL thickness and quadriceps ACSA)

Abbreviations: ACSA = anatomical cross-sectional area, AF = atrial fibrillation/flutter, AKI = acute kidney injury, CKD = chronic kidney disease, cm = centimetres, CVD = cerebral vascular disease, DM = diabetes mellitus, FBC = full blood count, HTN = hypertension, IHD = ischaemic heart disease, kg = kilogram, LFI = liver frailty Index, LFTs = liver function tests, SBP = spontaneous bacterial peritonitis, VL = vastus lateralis

3.5.2 Physical activity

Physical activity was objectively assessed using the reliable and valid wrist-worn triaxial accelerometer GENEActiv® (Activinsights, Cambridge UK) (**Figure 3.2**) (254). Two weeks prior

to each visit, participants were either given or posted the GENEActiv® watch device and asked to wear it on their non-dominant hand 24 hours/day for up to 14 days. Accelerometers were set to sample at a frequency of 10Hz and data were stored in 5-second (s) epochs. Participants returned their watches at their next visit when the watches were initialised and data downloaded in binary format using GENEActiv PC (version 3.1).

Figure 3.2 GENEActiv device worn by participants
Note: No activity feedback data available on the screen.

The GENEActiv.bin files were extracted, processed and analysed using open-source R-package, GGIR (v1.11-0 http://cran.r-project.org) (255). Detection of non-wear and sustained abnormally high values, autocalibration, using local gravity as a reference, and calculation of the average magnitude of dynamic acceleration (i.e. the vector magnitude of acceleration corrected for gravity [Euclidean Norm minus 1g]) in milli-gravitational units (m*g*), averaged over 5-s epochs were all included in the signal processing (256). Participants were excluded if there were <3 days of valid wear (defined as >16 hours/day), if wear data was not present for each 15 minute period of the 24 hour cycle, or if the post-calibration error was greater than 10m*g* (256).

The following basic physical activity outcomes were collated: time spent inactive, in light, moderate and vigorous intensity (minutes), and total time spent at moderate to vigorous intensity (**Table 3.3**). To enable deeper analysis of physical activity profiling, these additional outcomes were collated: average acceleration; intensity gradient; accumulation of acceleration of the most active X minutes (MX metrics, where X is the number of minutes); (M2, M5, M10, M15, M30, M60, M120 and M480 minutes); most active continuous X minutes (CM1, CM2, CM5, CM10, CM15, CM30, CM60, CM120, CM480 minutes) (**Table 3.4**).

A full explanation of each of these metrics and how they are calculated is provided in **Table 3.3** and **Table** 3.4. Furthermore, visualisation of the metrics, intensity gradient, accumulation of most active minutes, and most active continuous minutes are shown in **Figure 3.4** and **Figure** 3.4.

Table 3.3 Explanation of basic physical activity metrics

Metric	Unit	Description	Understanding the metric
Total moderate to vigorous physical activity	mins	The amount of time the participant spends at an intensity above the cut point for moderate intensity i.e. >100m*g* per day. It is calculated for all valid days and then averaged to give a recording of average minutes per day.	This indicates how many minutes a day the participant spends in either moderate or vigorous intensity activity as defined by a pre-determined cut point (for the purpose of this study 100m*g*)
Inactive	mins	The amount of time the participant spends at an intensity below the cut point 40m*g* intensity. It is calculated for all valid days and averaged to give a recording of average minutes per day.	This indicates the number of minutes a day the participant is inactive or sedentary.
Light intensity	mins	The amount of time the participant spends at an intensity between the cut points of 40-100m*g* intensity. It is calculated for all valid days and averaged to give a recording of average minutes per day.	This indicates the number of minutes a day the participant spends in light intensity activity.
Moderate intensity	mins	The amount of time the participant spends at an intensity between the cut points of 100-400m*g* intensity. It is calculated for all valid days and averaged to give a recording of average minutes per day.	This indicates the number of minutes a day the participant spends in moderate intensity activity .
Vigorous intensity	mins	The amount of time the participant spends at an intensity above a cut point of 400m*g* intensity. It is calculated for all valid days and averaged to give a recording of average minutes per day.	This indicates the number of minutes a day the participant spends in vigorous intensity activity.

Abbreviations: m*g*=milli-gravitational units, mins = minutes

Table adapted from Dawkins et al 2021 (257)

Table 3.4 Explanation of deeper analytical physical activity metrics

Metric	Unit	Description	Understanding the metric
Average acceleration	m*g*	The average acceleration for each valid 24hr day is calculated and then averaged across all valid days. It is not protocol or population specific. (228)	This indicates the overall 24hr total physical activity.
Intensity gradient	N/A	Total 24hr time for all participants is 1440 mins. Intensity gradient describes the curvilinear relationship between intensity and time spent at that intensity. The majority of a 24hr period is spent at low intensity (0-25m*g*). As time intensity increases time accumulation drops off, for example, minimal amount of time would be accumulated at high intensities (>1000m*g*). To obtain an intensity gradient, the curvilinear relationship is converted to a straight line relationship by taking the natural log of the two wide ranging quantities of intensity and time, i.e. the midrange of each of the intensity bins (e.g., 0-25m*g* bin=12.5m*g*) and the time accumulated in each bin. The R^2 (indicative of the goodness of fit of the linear model), gradient and constant (y-intercept) of the linear regression equation for each participant is recorded. This provides a negative gradient which reflects the drop in time accumulated as intensity increases. A visual explanation can be found in **Figure 3.3**. The gradient is calculated for each valid 24hr day and averaged across all valid days. (228)	This indicates the proportion of the day spent at higher intensities. A more positive intensity gradient indicates a higher proportion of the day spent at higher intensities and the more negative the intensity gradient the greater the proportion of the day is spent at lower intensities. See **Figure 3.3** for a visual explanation.
Accumulation of most active time periods	m*g*	The minimum acceleration for the most active X minutes accumulated, where X = time. The activity can be accumulated at any time point during the 24hrs, i.e. it	The intensity (acceleration) the participant accumulates for a set time (X) period. For example, if a participant's M10 is 150m*g* then they spend a total of 10 minutes a day at an

		does not need to be continuous or in specific time bouts and is expressed as MX. For example, minimum acceleration for most active 2mins = M2. The MX metric is calculated by sorting each 5s epoch of acceleration across the day in descending magnitude. The minimum acceleration for the most active X minutes corresponds with the top X minutes in the descending list. For example M2 would correspond with the top 2 mins of acceleration and shows the intensity that the person exceeded for a total of 2 mins across the day. The gradient is calculated for each valid 24hr day and averaged across all valid days.	intensity higher than 150m*g*. This MX values can then be used to compare to other cut points in retrospect For example, an M10 of 200m*g* would indicate that they achieve, on average, 10 minutes a day at MVPA if the MVPA cut point was 100m*g*. However, if the MVPA cut point was 200m*g*, then they would not have reached the threshold. This enables wider comparison of data between patient populations and where different cut points have been used, without changing the metric. Radar plots are a visually effective way to display these metrics. Examples of which can be found in **Figure 3.4**.
Accumulation of most active continuous time periods	m*g*	The minimum acceleration accumulated for the most active continuous X minutes, where X = time. The activity needs to be continuous, but not for specific time bouts. It is expressed as CMX. For example, minimum acceleration for most active continuous 2mins = CM2. The CMX metric is calculated by sorting each 5s epoch of acceleration across the day in descending magnitude. The minimum acceleration for the most active continuous X mins corresponds with the top X continuous mins in the descending list. For example CM2 would correspond with the top continuous 2 mins of acceleration and shows the intensity that the person exceeded for a continuous 2 mins across the day. The gradient is calculated for each valid 24hr day and averaged across all valid days.	The intensity (acceleration) the participant accumulates continuously for a set time (X) period. For example, if a participants CM10 is 150m*g* then they participated in activity continuously, on average, for 10 minutes a day at an intensity higher than 150m*g*. This CMX values can then be used to compare to other cut points in retrospect as described above. Radar plots are a visually effective way to display these metrics. Examples of which can be found in **Figure 3.4**.

Table adapted from Dawkins et al 2021 (257)

Abbreviations: hr(s)=hour(s); m*g*=miligravitational units; mins=minutes; MVPA=moderate to vigorous physical activity

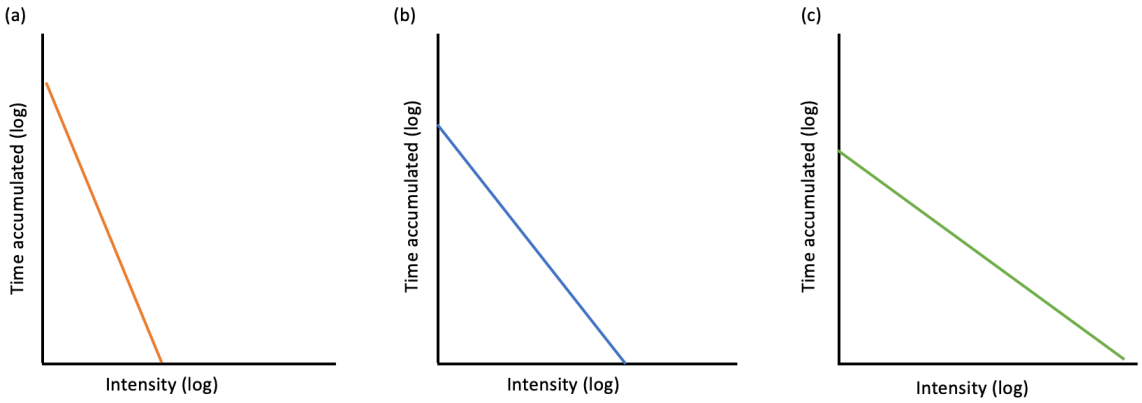


Figure 3.3 An example of how; (a) low, (b) moderate and (c) high intensity gradients may present

(a) is a steeper, more negative (lower) gradient with a higher constant (y-intercept). This shows a steep drop in time accumulated as intensity increases, demonstrating a poorer intensity profile.

(b) is a less negative gradient with a slightly lower constant, suggesting a moderate intensity profile.

(c) has a shallow, less negative (higher) gradient with a lower constant (y-intercept) showing more time spread across the intensity range. It represents a better intensity profile with a greater proportion of time spent at higher intensities.

Figure adapted from Rowlands et al. (2018)

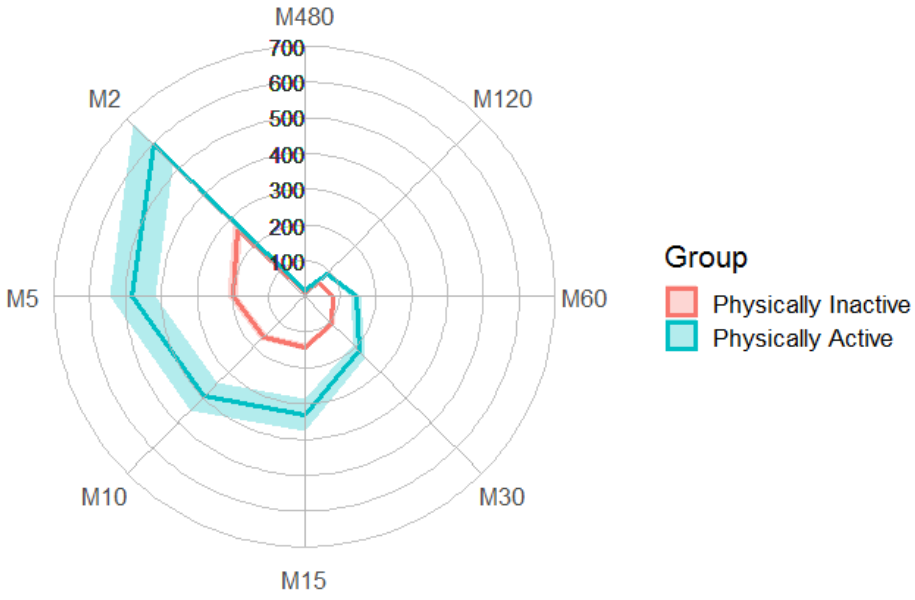


Figure 3.4 An example of a radar plot demonstrating typical patterns of those who are physically active (green) and those who are physically inactive (red)

Each MX represents a time point. I.e. M2 = 2mins, M15=15 minutes etc. of accumulated or continuous intensity, depending on raw data used. Each circle indicates a level of intensity, starting at 0 milli-gravitational units (m*g*) in the centre to 700m*g* on the outskirts of the radar plot.

3.5.3 Physical function/strength

Physical function was assessed using the Liver Frailty Index (LFI), the gait speed element of the Short Physical Performance Battery (SPPB) and isokinetic dynamometry, which were all completed as part of the ESICID study.

3.5.3.1 Liver frailty index

The LFI was calculated (available at: http://liverfrailtyindex.ucsf.edu) following completion of its three performance-based measures; hand grip strength, timed chair stands and balance testing (136). Each measure was assessed using the same protocol outlined in Chapter 2. Each patient was categorised as either robust (LFI <3.2), pre-frail (LFI ≥3.2-4.4) or frail (LFI ≥4.5), according to their index.

3.5.3.2 Gait speed

As part of the SPPB, participants were asked to walk a four metre course twice at "their own walking pace"(258). Each four metre walk was timed using a stopwatch and the fastest speed was used for analysis. Gait speed was calculated by dividing the fastest walk time by four and recorded as metres per second.

3.5.3.3 Isokinetic dynamometry

Isokinetic dynamometry has been established as the gold standard for evaluating muscle strength of the lower limb (259). It is a reliable and valid method that can measure maximal torque throughout the whole range of motion (260). For this study, maximal unilateral knee extension (via peak torque) of the non-dominant leg was conducted using the Biodex Medical System 3 (Biodex Medical Systems, New York, United States). Participants were seated with

hips and knees at 90^{o} and straps were placed across the chest, pelvis and thigh, to secure the participant in place. The pivot was set to the lateral condyle of the femur and the dynamometer arm was fixated at the participant's ankle, to allow for maximal range of motion assessment (261). While the participant's leg was left loose, measurement of the leg weight was completed by the dynamometer, so that gravity elimination could be provided by the dynamometer during the test. Range of movement was set from 90^{o} to 0^{o} (0^{o} representing full extension) and three isokinetic muscle actions were completed at 60$^{o}\cdot s^{-1}$. The procedure was explained and the participant conducted three repetitions of warm-up and understanding. Following this, the participant was instructed to fully extend and flex the knee as "hard and fast" as possible five times (261). During the test each participant was given the same verbal commands by a member of the research team. The machine calculated the highest recorded peak torque during five completed contractions, which was then used within statistical analysis.

Isokinetic dynamometry was also used to record dominant hand-grip strength. The method used to measure hand-grip strength is outlined in Chapter 2 as a component of the LFI.

3.5.4 Muscle mass

3.5.4.1 Thigh muscle ultrasound

Ultrasonographic imaging, using the Esoate MyLab Alpha (Genoa, Italy) point of care ultrasound machine (4.6cm probe, SL1543, 13-4Mhz scanning frequency), of the quadriceps muscle group on the dominant leg was completed. Following a minimum of 10 minutes rest, participants lay semi supine with the knee in full extension (262).

3.5.4.1.1 Vastus lateralis thickness

To measure vastus lateralis (VL) thickness, the transducer was placed longitudinally at 50% of femur length (as measured from the greater trochanter to the lateral knee joint space), where the anatomic cross sectional area (ACSA) is at its maximum (263). Care was taken by the operator to align the transducer to the fascicle plane and apply as little pressure as possible for optimal view. A semi-automated FIJI macro tool (Simple Muscle Architecture Analysis version 1.7) was completed, using ImageJ software (National Institute of Health, USA) to estimate VL thickness (**Figure 3.5**) (264). Analysis was completed in triplicate and the mean average was used.

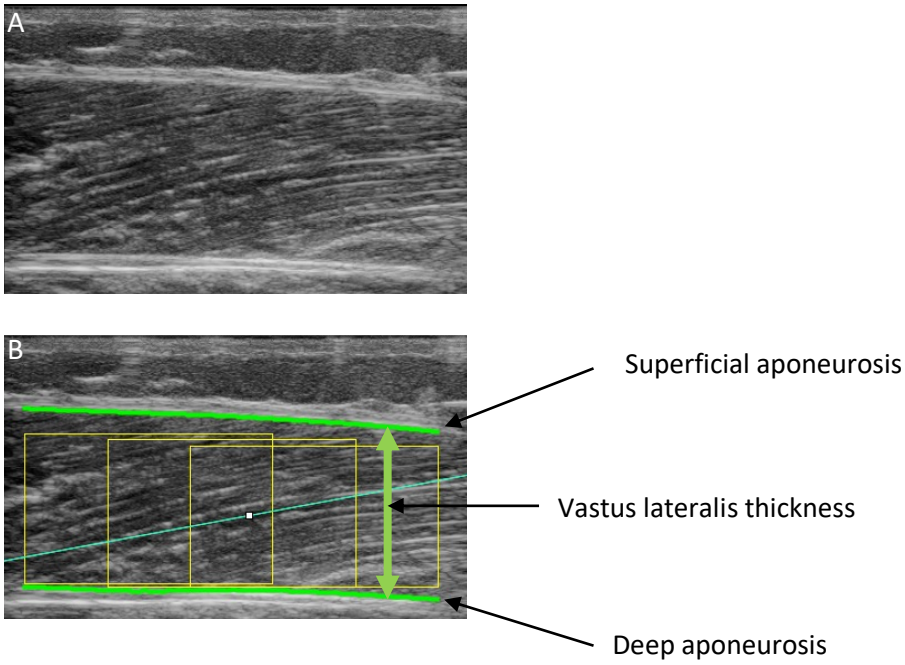


Figure 3.5 Ultrasound image of vastus lateralis
A semi-automated FiJi macro tool (Simple Muscle Architecture analysis) was completed using ImageJ software (National Institute of Health, USA). Analysis was completed in triplicate with estimated vastus lateralis muscle thickness, fascicle length and pennation angle recorded. Vastus lateralis thickness was only used for the purpose of this thesis.

3.5.4.1.2 Quadriceps anatomical cross-sectional area

With the participant positioned as above, two extended field of view (EFOV) images were taken at 50% femur length (measured as above) in the transversal plane (265). EFOV has been shown to be a reliable and valid tool for assessing quadriceps anatomical cross sectional area (ACSA) when compared with magnetic resonance imaging (MRI) or computed tomography (CT) (266-268). The mean of both views were calculated and used for statistical analysis.

A B

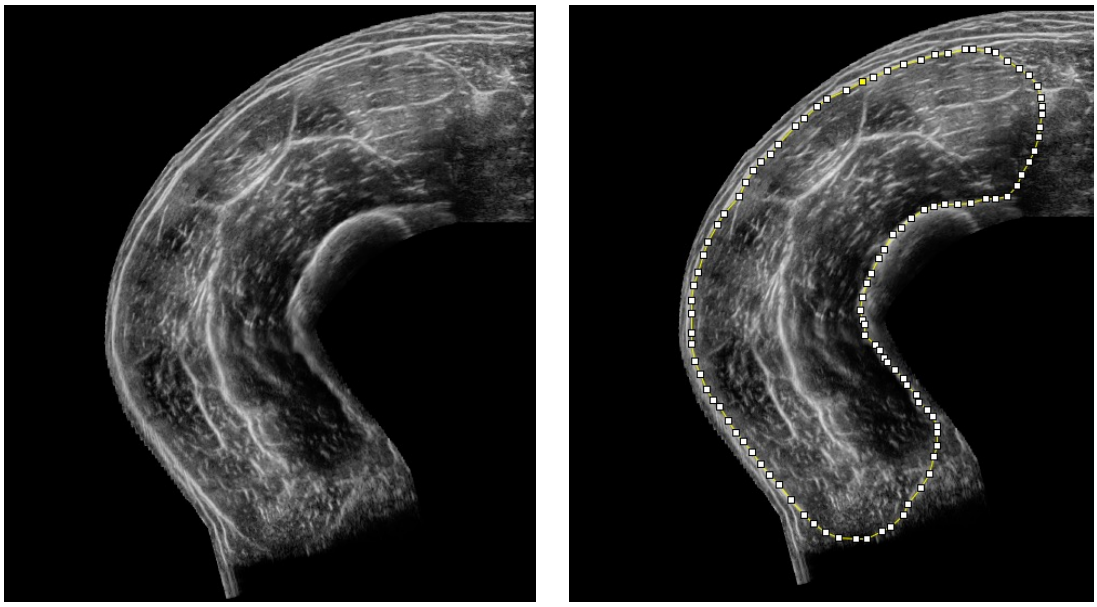


Figure 3.6 Anatomical cross-sectional area of quadriceps
A=Extended field of view ultrasound image of quadriceps.
B=Outline drawn around quadriceps to enable calculation of the mean anatomical cross-sectional area using the software, ImageJ (National Institute of Health, USA).

3.5.5 Others

Many other outcomes/parameters were collated as part of the ESCID, but were not incorporated in this thesis including; anthropometry (triceps skin fold, mid-arm muscle circumference), quality of life questionnaires (disease specific and short-form-36), quadriceps and lumbar vertebra level 3 MRI, muscle histology, inflammatory cytokines, and sleep.

3.6 Statistical Analysis

All quantitative data was entered into a purpose-designed database and exported for statistical analysis in statistical software such as SPSS (version 28.0.0.0) or GraphPad Prism (version 9.2). Specific statistical analysis are described within each of the subsequent chapters.

3.7 Discussion

The ESICID study was designed to describe the prevalence of sarcopenia and the impact of molecular and lifestyle factors on the chronic inflammatory diseases; ESLD, IBD and IA. By 16th June 2021, 102 participants (53 with ESLD) were recruited to the study. This was the first study to deeply explore muscle health in those with ESLD. Novel protocols for the assessment and analysis of muscle mass via MRI and thigh muscle ultrasound in ESLD were used, where research is currently scarce (234). Furthermore, new protocols were developed, including the obtainment of muscle biopsies, which has been avoided in the past due to the risk of coagulation related complications in ESLD. I am a co-author on the paper for this protocol, which can be found at: https://pubmed.ncbi.nlm.nih.gov/35242045/ (269). The analysis of physical activity in the ESCID study, and included in Chapters 4 and 5, are not only novel within ESLD, but also within the field of physical activity, demonstrating the originality and innovative nature of the work conducted within this thesis.

There were however, challenges throughout the study. Firstly, patients with ESLD have a high degree of disease burden and fatigue easily. Early identification of decline was needed with regular communication with the Liver on call Registrar and/or Consultant. Secondly, in March

2020, the study was suspended due to COVID-19 and several members of the ESCID research team, including myself, were re-deployed to COVID-19 intensive care unit. This meant that many patients did not complete their visit 4 and the healthy control data was not collated until the following year (May 2021), once restrictions on studies were lifted. Even then, healthy control recruitment was limited to staff at either the University or the QEHB to avoid putting members of the public at any undue risk.

CHAPTER 4:
PHYSICAL ACTIVITY PATTERNS IN PATIENTS WITH END-STAGE LIVER DISEASE: AN OBSERVATIONAL, PROSPECTIVE, CONTROLLED STUDY

4.1 Introduction

Physical activity is any bodily movement, produced by skeletal muscle, that requires energy expenditure (213). Physical inactivity, defined as not meeting the minimum physical activity to preserve physical fitness and health (270), results in a significant reduction in muscle protein synthesis, increased protein breakdown (proteolysis) and subsequent muscle fibre atrophy (271). Subsequently, these lead to loss of muscle mass, strength and function, which contribute to physical frailty. Clinically, physical inactivity increases the risk of developing liver disease, accelerates liver disease progression, and increases the risk of overall and liver-related mortality (272). For patients with end-stage liver disease (ESLD), physical inactivity is associated with liver transplant (LT) waiting list mortality (273), yet research investigating physical activity patterns in this cohort is relatively unexplored.

A few studies have subjectively assessed physical activity using the questionnaires International Physical Activity Questionnaire (IPAQ) and the Karnofsky activity scale in patients with ESLD (215, 274, 275). Whilst these subjective studies highlight the reduced participation in physical activity and associated impact on post-hospital discharge mortality, they do not provide an accurate, objective description of the 24-hour per day physical activity pattern in patients with ESLD. Furthermore, subjective measures of physical activity have been shown to be unreliable, demonstrating the need for objective physical activity monitoring through the use of wearable devices, such as accelerometers (273). Two studies to date have used objective measures to investigate physical activity using wrist worn accelerometers (273, 276). Whilst a profound level of physical inactivity was demonstrated in patients with ESLD (n= 93), findings were limited to a United States (US) population and were

constrained to basic outcomes such as, step counts, overall activity and time spent in specific intensity activities such as moderate to vigorous physical activity (MVPA) and/or sedentary time (273, 276). Although such outcomes are simple to apply and lend themselves to comparison with public health guidelines, they only provide physical activity profiling for small proportions of the day. Moreover, they rely on specific cut-off points which limit comparability between datasets (229).

In recent years, the use of physical activity and/or exercise interventions to reduce physical inactivity and reverse physical frailty has gained in popularity (178, 182, 183, 185, 190-192, 277). Yet, heterogeneity between studies, particularly in relation to frequency, intensity, time and type (FITT) of activity and variance in reported adherence, has limited the implementation of these findings into clinical practice. Therefore, a thorough understanding of physical activity patterns through intricate and accurate measurement is crucial to design and target effective future interventions. This may be achieved by utilising data-driven metrics such as average acceleration and intensity gradient (described in section 3.5.1). The use of such metrics preserves the continuous nature of accelerometer metrics, enables intricate analysis of physical activity profiling and allow for post-hoc analysis in relation to any pre-defined cut-off points (229).

Hence, this chapter aims to provide the first in depth analysis of 24-hour physical activity profiling of a UK based cohort with ESLD, and compare this to age/sex matched healthy controls (HC). The following aims will be addressed:

Aim 1: Determine overall physical activity and time spent at different activity intensities (i.e. sedentary, light, moderate, vigorous) between those with ESLD and HC.

Aim 2: Determine differences in total volume (average acceleration) and distribution of daily activity intensity (intensity gradient) between ESLD and HC.

Aim 3: Determine differences in accumulation of, and/or continuous bouts of intensity for given time periods between those with ESLD and HC.

4.2 Methods

4.2.1 Recruitment and assessment of physical activity

Participants were recruited and physical activity assessed as part of the prospective, case-control ESICID study. Physical activity data was obtained for analysis using the first available visit data (prior to a LT) that had ≥3 valid wear days as described in section 3.3.2 and 3.5.1.

4.2.2 Statistical analysis

All continuous data are presented as either mean (standard deviation [SD]) or median (inter quartile range [IQR]) depending on the distribution of normality, determined by a D'Agostino and Pearson test. All categorical data are presented as a number (n) and percentage (%). Differences between two groups (ESLD and HC) were analysed using either an unpaired two-tailed t-test or Mann Whitney test, depending on whether the data were parametric or non-parametric, respectively. Level of significance was set at $p<0.05$ for all statistical tests. Radar plots were generated in Rstudio (version 2.0) using the github code: https://github.com/Maylor8/RadarPlotGenerator (229). Radar plots provide meaningful visual comparisons of accelerometer-assessed physical activity (**Figure 3.4**). Chapter 3 gives a detailed overview of how to interpret radar plots, but in short:

- The circumference represents increasing amounts of the most active accumulated time widows ranging from 2 minutes (M2) round to 480 minutes (M480), in an anti-clockwise direction.
- The distance of the data point from the centre of the circle outwards represents the minimum average acceleration (measured in milli-gravitational units [m*g*]) reported for each time period.
- Blue dashed lines represent accelerations which are indicative of certain levels of intensity (i.e. 100m*g* = slow walk, 250m*g* = brisk walk and 400m*g* = slow run).

4.3 Results

4.3.1 Participant demographics

43/53 participants with ESLD were included in the analysis, with 10 patients excluded from the analysis due to <3 days of valid accelerometer wear. 28/43 (65%) were male, median age 56.0 (IQR 50.0-60.0) years and median BMI was 29.2 (IQR 24.2-32.2) kg/m^2. Liver disease severity was signified by a median UKELD 56.0 (IQR 50.0-60.0), with 19/43 (44%) and 9/43 (21%) presenting with refractory ascites (i.e. resistant to diuretic treatment) and/or hepatic encephalopathy (HE) (i.e. grade I/II west-haven, despite treatment with rifaximin/lactulose), respectively. Alcohol related liver disease (ArLD) was the most commonly reported disease aetiology (51%, n=23), followed by autoimmune liver diseases (AID) (30%, n=13), non-alcoholic fatty liver disease (NAFLD) (12%, n=5) and other (5%, n=2) (**Table 4.1**). In comparison, 17/18 age/sex matched HC, 56% (n=10) male, median age 47.0 (33.5-58.8) years, BMI 25.0 (4.2) kg/m^2, had accelerometery data of ≥3 valid days. The HC group reported no

chronic inflammatory conditions/medical comorbidities, were deemed recreationally active and did not participate in any structured exercise protocols prior to the study (**Table 4.1**).

Table 4.1 Demographics and clinical characteristics of participants with end-stage liver disease (ESLD) and healthy controls (HC)

Clinical characteristics	ESLD (n=43)	HC (n=17)
Age	56.0 (50.0-60.0)	47.0 (33.5-58.8)
Sex (male)	28 (65%)	10 (56%)
Disease Aetiology:		
ArLD	23 (53%)	-
NAFLD	5 (11%)	-
AID	13 (30%)	-
Other	2 (5%)	-
Bloods:		
Na mmol/L	137.0 (135-140)	140.0 (138.3-141.0)
Bilirubin mmol/L	34.0 (22.0-45.0)	12.0 (8.3-15.3)
INR	1.2 (1.2-1.3)	1.0 (0.9-1.0)
Urea mmol/L	5.4 (4.0-8.9)	5.2 (4.6-5.8)
Creatinine (umol/L)	74.0 (59.0-90.0)	79.0 (68.0-87.8)
EGFR	90.0 (68.0-90.0)	83.5 (72.8-90.0)
Hb g/dL	125 (116.0-133.0)	140.4 (12.7)
WCC $x10^9$	4.5 (3.6-6.7)	4.9 (4.4-6.3)
Ammonia mmol/L	59.0 (47.0-74.0)	-
Albumin mmol/L	34.0 (30.0-37.0)	42.0 (40.3-46.0)
Liver disease severity		
UKELD	52.0 (50.0-55.0)	-
MELD	11.0 (10.0-15.0)	-
CPS		
A	11 (25.6%)	-
B	20 (46.5%)	-
C	4 (9.3%)	-
Previous variceal haemorrhage	5 (11%)	0
PVT	0 (0%)	0
Refractory HE	9 (21%)	0
Refractory ascites	19 (44%)	0
Metabolic Comorbidities:		
BMI kg/m^2	29.6 (24.2-34.2)	24.4 (22.1-27.7)
Hypertension	6 (14%)	0
Type 2 Diabetes	9 (20%)	0
Atrial Fibrillation	0 (0%)	0
IHD	2 (5%)	0
Smoking history		
Non-smoker	23 (52%)	17 (100%)
Ex-smoker	19 (43%)	0
Current smoker	2 (5%)	0

Data presented median (IQR) or n(%) (categorical data)
-not applicable
Abbreviations: AID = Autoimmune disease, ArLD = Alcohol related liver disease, CPS = Child's Pugh Score, EGFR = estimated glomerular filtration rate, g/dL = grams per decilitre, Hb = haemoglobin, HE = hepatic encephalopathy, IHD = ischaemic heart disease, INR = international normalised ratio, IQR = interquartile range, kg/m^2 = kilograms per metre squared, MELD = model for end-stage liver disease, mmol/L = milli-molecules per litre, Na = sodium, NAFLD = non-alcoholic fatty liver disease, PVT = portal vein thrombosis, UKELD = United Kingdom model for end-stage liver disease, WCC = white cell count, umol/L = micromole per litre

4.3.2 Aim 1: Comparison of twenty-four hour activity cycles between ESLD and HC

Overall, participants with ESLD spent a greater proportion of their 24-hour day inactive (mean (standard deviation[SD]) 798(128.5) vs 690(79.9) mins, p=0.005) (**Figure 4.1**). They also spent significantly less time participating in MVPA (mean(SD) 44.0(38.6) vs 105(40.9) mins, p<0.0001), when compared to HC (**Figure 4.2**). When MVPA was broken down, this significant difference between ESLD and HC was emulated in time spent at both moderate intensity physical activity (MIPA) (mean(SD) 43.1(46.3) vs 97.5(37.7) mins, p<0.0001) and vigorous intensity physical activity (VIPA) (mean(SD) 1.1(1.5) vs 7.8(8.4) mins, p<0.0001), respectively (**Figure 4.1**). There was no significant difference between minutes spent at light intensity between groups (mean(SD) 134.5(61.3) vs 158.0(45.2) mins, p=0.19).

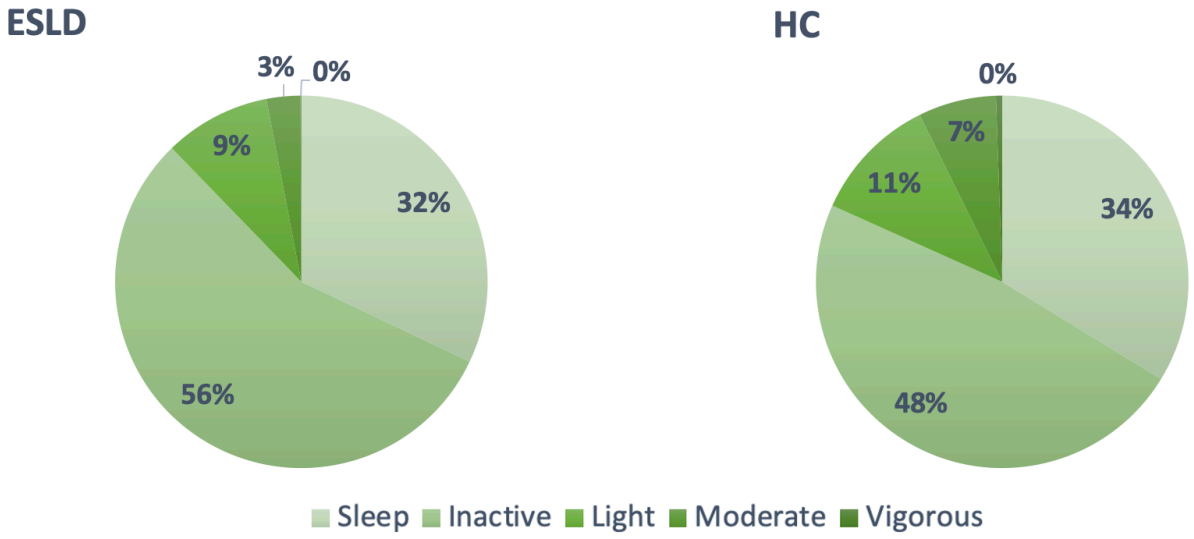


Figure 4.1 Pie charts comparing percentage of 24-hour period spent sleeping, inactive, in light, moderate and vigorous intensity activity in ESLD (left) and Healthy Controls (HC, right)

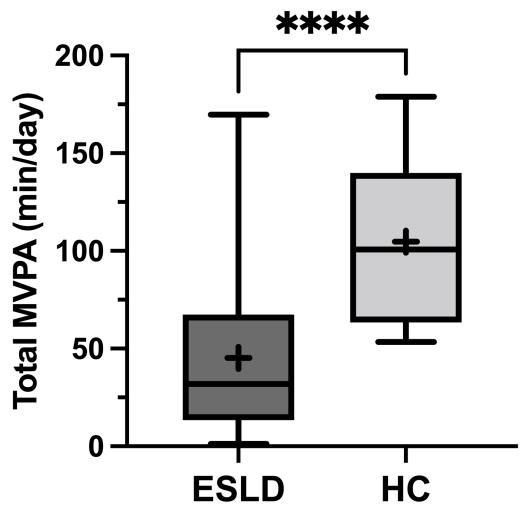


Figure 4.2 Graph comparing average total time in minutes spent participating in moderate to vigorous physical activity (MVPA) in ESLD and HCs
Note: Total MVPA was defined as activity at an intensity >100m*g*
Data expressed as median (central horizontal line), 25th and 75th percentile (box) and the minimum and maximum (horizontal line).
+mean
****p<0.0001

4.3.3 Aim 2: Difference between overall volume and intensity distribution between ESLD and HC

The total volume of physical activity (average acceleration) completed by participants with ESLD was significantly less than HC (mean(SD) 17.8(7.5) vs 29.2(8.9)m*g*, p<0.0001). Additionally, the distribution of activity (intensity gradient) was significantly less in the ESLD group with the HC participating in a greater range of activity intensities throughout the day (mean -2.82 vs -2.40, p<0.0001) (**Figure 4.3**).

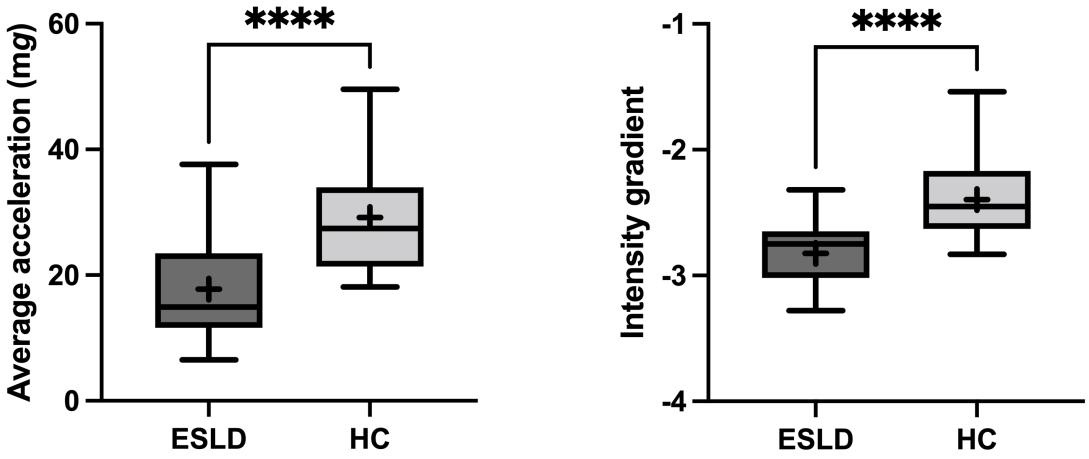


Figure 4.3 Graphs comparing average total volume of activity (average acceleration) and the average distribution of daily intensity (intensity gradient) in ESLD and HC

Note: The higher the intensity gradient, the greater the distribution of activity over inactive, light, moderate and vigorous intensities

Data are expressed as median (central horizontal line), 25th and 75th percentile (box) and the minimum and maximum (horizontal line).

+mean

****p<0.0001

Abbreviations: m*g*=milli-gravitational units

4.3.4 Aim 3: Comparison of average intensity for most active accumulated time periods between ESLD and HC

The average accumulated intensity of the most active time periods 2, 5, 10, 15, 30, 60, and 120 minutes were significantly less in ESLD compared to HC (**Table 4.2**). Over a 24-hour period, the ESLD group accumulated 2 minutes of activity at an intensity which is indicative of a brisk walk (250m*g*), whereas the HC accumulated approximately 20 minutes of activity for the same intensity (Error! Reference source not found.).

Table 4.2 Average intensity for most active accumulated time periods of the day

	Accumulated Intensity (mean (SD))		
Time Period (mins)	**ESLD (m*g*)**	**HC (m*g*)**	**P-value**
2 (M2)	263.0 (121.2)	597.9 (330.2)	<0.0001
5 (M5)	201.2 (88.8)	480.7 (265.0)	<0.0001
10 (M5)	161.5 (68.4)	396.2 (216.2)	<0.0001
15 (M15)	141.3 (58.3)	332.6 (187.8)	<0.0001
30 (M30)	109.8 (44.5)	216.0 (101.3)	<0.0001
60 (M60)	81.82 (33.40)	144.7 (57.3)	<0.0001
120 (M120)	53.54 (24.3)	90.5 (26.2)	<0.0001

Data expressed as mean(SD).
Unpaired two-tailed t-test or Mann Whitney test for parametric and non-parametric data respectively, were used to describe statistical difference between ESLD and HC for M120-M2.
Abbreviations: ESLD = end-stage liver disease, HC = healthy controls, M = minutes, m*g* = milli-gravitational units, SD = standard deviation

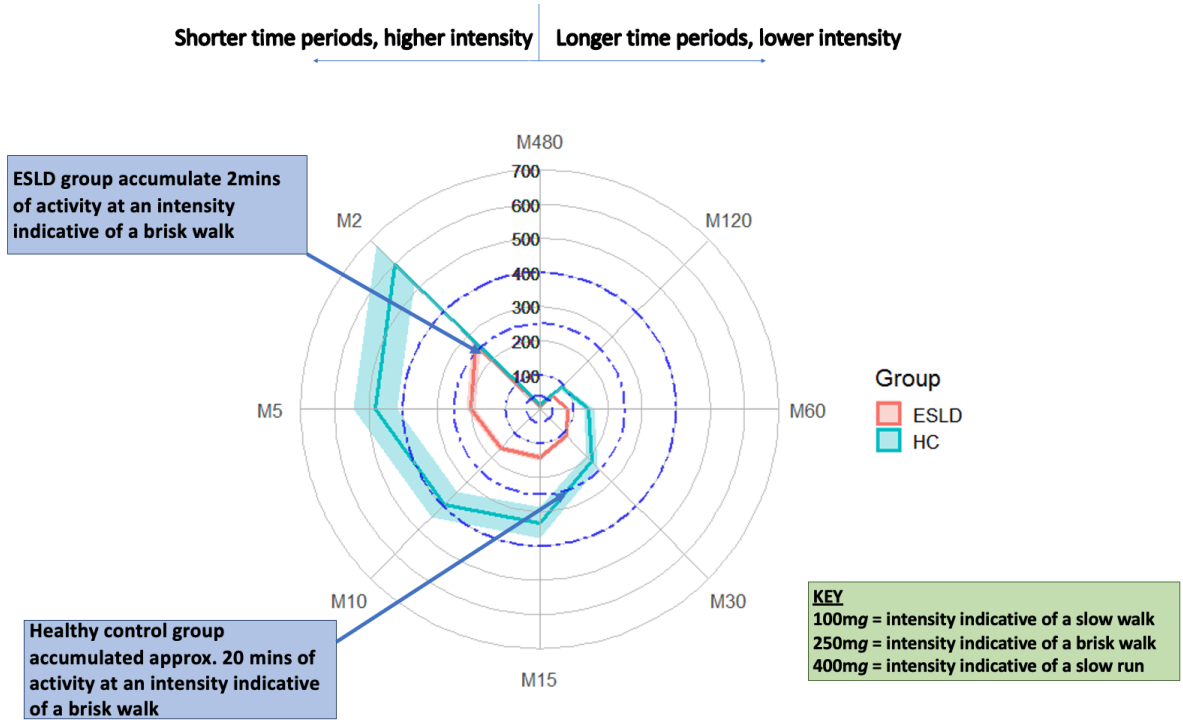


Figure 4.4 Radar plot illustrating the minimum average acceleration for the most active accumulated time periods for ESLD and HC
Note: Time periods reported included: 480mins (M480), 120mins (M120), 60 mins (M60), 30mins (M30), 15mins (M15), 10mins (M10), 5 mins (M5), 2mins (M2), 1min (M1).
Abbreviations: m*g*=milli-gravitational units, M = minute(s)

4.3.5 Aim 3: Comparison of average intensity for most active continuous time periods between ESLD and HC

The average acceleration for the most active continuous time periods (1, 2, 5, 10, 15 and 30 mins) was significantly less in ESLD participants compared to HC (**Table 4.3**). Specifically, during their most active continuous one minute, participants with ESLD moved significantly less intensively (313.4m*g* vs 691.9m*g*, p<0.001) than HC and did not sustain MIPA (>100m*g* (intensity indicative of a slow walk)) for longer than one minute. In contrast, on average, HC participated in continuous MIPA for 30 minutes (Error! Reference source not found.).

Table 4.3 Average intensity for most active continuous time periods of the day

	Most active continuous intensity (median, IQR)		
Time Period (mins)	**ESLD (m*g*)**	**HC (m*g*)**	**P-value**
1 (CM1)	313 (198-395)	612 (395-1066)	<0.0001
2 (CM2)	66.9 (47.1-102.0)	166.4 (120.9-375.4)	<0.0001
5 (CM5)	54.15 (34.5-81.8)	161.5 (104.1-313.4)	<0.0001
10 (CM10)	43.7 (30.4-63.7)	124.2 (88.7-280.0)	<0.0001
15 (CM15)	36.8 (23.0-51.9)	115.5 (74.2-264.6)	<0.0001
30 (CM30)	26.8 (15.0-41.7)	79.3 (49.8-430.8)	<0.0001

Abbreviations: CM = continuous minutes, ESLD = end-stage liver disease, HC = healthy controls, IQR = interquartile range, m*g* = milli-gravitational units, mins = minutes

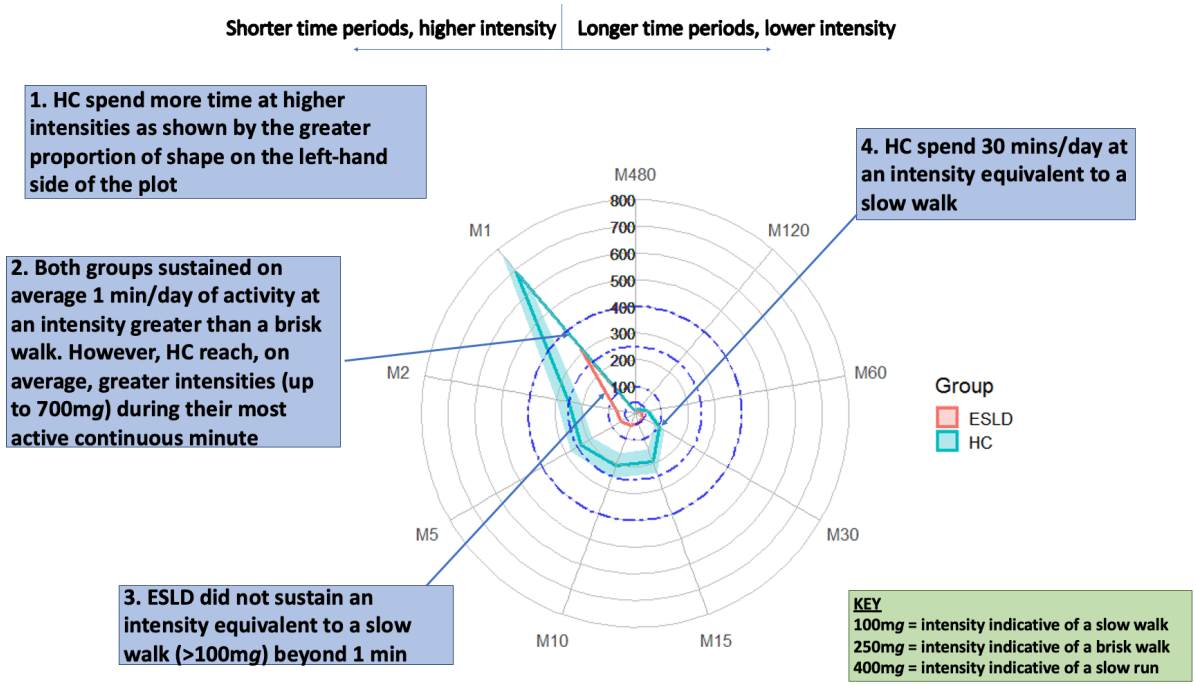


Figure 4.5 Radar plot illustrating metrics for minimum average acceleration for the most active continuous time periods for ESLD and HC

Note: Time periods reported included: 30mins (M30), 15mins (M15), 10mins (M10), 5 mins (M5), 2mins (M2), 1min (M1).

Abbreviation: m*g*=milli-gravitational units, M = minute(s)

4.4 Discussion

Physical inactivity is one of the main driving factors of physical frailty in ESLD. In recent years multiple studies have investigated the use of physical activity and exercise interventions to improve measures of physical frailty in ESLD (178, 182, 183, 185, 187, 190-193, 277). However, small sample sizes and heterogeneity of interventions between studies has limited application of findings into practice (206). To inform the design of future physical activity/exercise-based interventions, a better understanding of current physical activity patterns in patients with ESLD is urgently needed. The gaining popularity of wrist worn accelerometer devices to determine physical activity in a variety of populations has filtered into the literature of those with ESLD, but there remains a paucity of data, in particular capturing the full 24-hour in their daily lives and incorporating intensity modelling. Like many previous non-liver studies, overall activity reporting in the current study has been used with the common descriptions of total daily MVPA and time spent in the pre-defined categories of light, moderate, vigorous and sedentary activity.

4.4.1 Key findings

On average, participants with ESLD were more inactive/sedentary (56 vs 48% per day) and participated in less total MVPA (3 vs 7% per day) when compared to healthy controls. Participants with ESLD also accumulated less volume of activity throughout the 24-hour period, with less of their day spent in activities requiring higher intensity activity. More specifically, not only did participants with ESLD spend less total time in MVPA, but they accumulated activity at a much lower intensity and continuous MIPA was not maintained for as long as the HC group.

4.4.2 Aim 1: Sedentary time

The overall physical activity patterns, particularly time spent in MVPA, reported in our study are similar to those found by others within the field (273, 276). However, there was a distinct difference in the described sedentary times. Dunn and colleagues (2016) reported longer sedentary times (75 vs our 56% of wear time) in 53 patients listed for liver transplantation. This may be due to the time period differences in data collection methods between studies. Dunn and colleagues (2016) instructed their participants to wear their accelerometers during waking hours only and collated data with a minimum of 10-hours valid wear time, whereas our data is based on 24-hours with a minimum of 16-hours valid wear time (273). Therefore, our calculations of sedentary time are over a greater time period and include periods where the participant may have been active in between periods of sleep-wake cycles, which may have contributed to the lower times reported here.

Similar to the methods used in our study, Handalzalts and colleagues, instructed 40 participants with cirrhosis to wear their accelerometers for 24-hours a day over a 7-day period (276). Interestingly, Handalzalts and colleagues also reported a much higher percentage sedentary time of 89% and 85% in frail (n=10; defined as ≥3 on the Fried Frailty Index), and non-frail (n=30) cirrhotic patients, respectively. These long sedentary times may be explained by the lack of discrimination between sleep and sedentary time analysis by the authors. If sleep and sedentary time are combined within our study, participants with ESLD were comparable with 88% of their day spent either sedentary or sleeping.

The relationship between sedentary time and clinical outcomes in patients with ESLD is yet to be investigated. Nevertheless, the World Health Organisation (WHO) state that adults

should minimise time spent sedentary as this has many health benefits (213). In particular, those who spend less than 9.5 hours/day (<40%) sedentary have a significantly lower risk of death (278). Overall, although the ESLD spent significantly more time sedentary, both groups spent a large proportion of their day sedentary (13.5 and 11.5 hours, respectively), putting both groups into the high risk category for overall mortality. The significant amount of time spent sedentary in the HC group, may be explained by the recruitment of mainly academic adults and the recruitment period for HC taking place within the COVID-19 pandemic (May 2021). Sedentary working patterns and UK Government restrictions on socialising and activity in groups likely contributed to this high sedentary time. Despite these biases towards sedentary behaviour within the HC group, participants with ESLD still spent significantly more time sedentary which further highlights the need for interventions to reduce sedentary time in this patient cohort.

4.4.3 Aim 1: Total moderate to vigorous physical activity

In addition to the recommended guidance to reduce sedentary time, the WHO states that healthy adults, and those with chronic diseases (i.e. diabetes, cardiovascular disease etc), should participate in a minimum of 150 minutes of moderate intensity or 75 minutes of vigorous intensity physical activity per week. Ideally, adults should aim for 300 minutes per week, as this provides additional health benefits, yet the health benefits beyond this point are unknown (213). The ESLD group participated in, on average, 309 minutes per week of MVPA. Although this was significantly less than the HC group (736 minutes of MVPA), it would appear that the WHO guidelines for total physical activity were just met. That being said, there is huge variability in cut-off points for MVPA within the literature (257). Migueles and colleagues, in a study comparing estimations of time spent in different physical activity

intensities in children with high BMIs across different age-appropriate cut-points, found 8-96% variation in participants meeting physical activity guidelines. They went on to conclude that *"it is not possible (and probably never will be) to know the prevalence of meeting physical activity guidelines based on accelerometery data"* (279).

This opinion is further supported by Troiano and colleagues who highlight the distinct differences between device-based measures and self-reported questionnaires (224). Accelerometer-based monitors quantify raw acceleration signals from physical activity-associated bodily motion, whereas self-report instruments quantify physical activity based on patterns or groupings of specific activities (i.e. sitting, standing, walking etc.). While both measures are valuable within the parameters of each assessment method (movement vs behaviour), the time difference and varying levels of aggregation make direct comparison challenging (224) and are not seen to be interchangeable (212). Considering all physical activity guidelines are based upon self-reported questionnaires, the comparison of our accelerometery data to current physical activity guidelines cannot be done and therefore adherence to WHO guidelines within this cohort remains unknown.

However, when comparing our data with other accelerometer based studies of physical activity in ESLD, there are similarities in findings. The 3% of wear time spent in MIPA in our study is consistent with those found by Dunn et al. (2016) and Handalzalts et al. (2022) who reported 4.9 and 0.8-2%, respectively (273, 276). Nevertheless, it is still difficult to compare these results with our study due to differences in data collection method and processing. For example, both Dunn and Handalzalts used the Sensewear Mini Armband (Jawbone Inc, San Francisco, CA), a bi-axial accelerometer device which records activity in "counts" (i.e. intensity

and magnitude of acceleration over a set time period) (211). Counts are calculated using proprietary algorithms which are developed and patented by manufacturers of the device, limiting comparability of data between devices, and ultimately between studies. In contrast, our study used the GENEActiv tri-axial accelerometer which records and stores raw acceleration data with no need to summarise into proprietary count-based epochs. Instead, a standardised measure of acceleration is used whereby the vector magnitude is calculated as the "Euclidean Norm Minus One" (ENMO) (i.e. summing the squared acceleration of each of the three accelerometer axes at each time point [i.e. Euclidean Norm] and then subtracting the gravitational component, which is 1g [1g=9.81m/s^2]). The use of such methods enables accurate comparisons between devices (220). Furthermore, rather than reporting in specific algorithm derived categories (i.e. light, moderate, vigorous) a raw acceleration is reported which can then be compared to specific cut-off points (220), allowing for generalisability of findings.

Despite these differences, and the small number of studies investigating objective physical activity monitoring, it would appear that patients with ESLD, and/or are listed for a LT have low levels of participation in MIPA and spend a large proportion of their time sedentary. Further studies with larger sample sizes and use of ENMO derived data are needed to harmonise activity data collection within ESLD.

4.4.4 Aim 2: Intensity modelling

As described above, whilst the analysis of overall activity and the various intensity categories has some merit, more intricate analysis of intensity modelling is needed to facilitate the investigation of shared associations of activity between populations and with health

outcomes (230). Through the use of ENMO derived data, this study analysed the following physical activity based outcomes: (a) average acceleration and intensity gradient; (b) accumulation of intensity for given time periods; (c) continuous intensity for a given time period. Average acceleration reflects the total amount (volume) of physical activity whereas intensity gradient reflects the distribution of activity throughout a 24-hour period. Higher intensity gradient values reflect a greater proportion of the day spent at higher activity intensities (230). Each measure explains distinct variance and have been shown to be equivalent for the three main research grade accelerometers (ActiGraph, GENEActiv, Axivity), benefiting accurate comparative analysis between non-liver disease studies (220). This study showed that participants with ESLD had significantly lower volume of activity and a significantly lower intensity gradient than the HC group. These findings indicate that the ESLD participated in less overall volume of activity and the HCs spent a greater proportion of their day at higher activity intensities.

The use of these measures in accelerometer analysis is very much in its infancy and as such, this is the first study to investigate such metrics within patients with ESLD. However, Dawkins and colleagues (2022) found that when predicting cardiometabolic risk, low volumes of total physical activity (average acceleration) with a low intensity gradient were associated with higher cardiometabolic risk in healthy individuals, whereas low total volume irrespective of intensity gradient was associated with cardiometabolic risk in those with chronic diseases, such as type II diabetes (280). These differences highlight the need to determine individual interactions of activity for specific diseases, as one cohort may not be comparable to another.

Moreover, further understanding of how these findings relate to clinical outcomes in ESLD would help guide intervention design and communication of patient acceptable physical activity messages for patients with ESLD. For example, if total volume for a 24-hour period was more important to health outcomes, then one might advise ESLD patients to "move more regardless of intensity" (i.e. move frequently even if this is at light intensity). Whereas if intensity suggests greater prediction of health outcomes then advice may centre around "when you move, move more intensively" (i.e. when walking the dog, increase the pace). To guide physical activity advice in ESLD, future research should investigate the relationship of average acceleration and intensity gradient with clinical outcomes such as physical frailty, waiting list mortality, hospital admission rates and health-related quality of life.

4.4.5 Aim 3: Accumulation of intensity for given time periods

Consideration of the average accumulation of activity intensity over set time periods provides context to the pre-categorised intensities. For example, for the GENEActiv device to recognise participation in MIPA, one must generate an acceleration between 100 and 400m*g*, at which point VIPA is recognised (281). With this in mind, the average activity accumulated between one and five minutes in this study would be categorised as MVPA minutes for both ESLD and HC. However, ESLD participants, on average, only participated in activity at an intensity between 100 and 250m*g (an intensity indicative of a slow to brisk walk)*, compared to the HC who, for the same time period, were participating at an intensity of 480 and 600mg (*an intensity indicative of running*). Likewise, between the time periods 5 and 30 minutes, ESLD activity intensity was only 100-150m*g*, whereas the HC participated in activity at an intensity of 250-400m*g* (**Figure 4.4**). Therefore, although participants with ESLD spent the recommended time in MVPA, the average intensity accumulated during these minutes was

significantly lower than the HC. Similarly, participants in either group may have participated in activity at an intensity of 99m*g*, yet this would not quantify MIPA, leading to miss-representation of average daily activity intensity accumulation.

These results were comparable to that found by Dawkins and colleagues (2021) who found that those with non-liver chronic diseases accumulated lower intensities for a given time period when compared to women with post-gestational diabetes and office workers (257). However, unlike our cohort with ESLD, the chronic disease group were able to accumulate intensities between 250 and 300m*g* between two and five minutes and maintained >200m*g* for up to 20 minutes. This higher accumulation of intensity may be due to the fact that people with frailty were excluded from the chronic disease cohort within this study, therefore participants were likely to be more functionally able than our group with ESLD. For example, one in five patients listed for a LT are considered frail and forty percent are functionally impaired (49, 50), data of which are supported by the findings in Chapter 2 of this thesis.

Additionally, it may be that lack of intensity/acceleration seen in our cohort with ESLD is driven by inactivity. Inactivity causes a reduction in AMPK activation and skeletal muscle glucose uptake, resulting in decreased muscle protein synthesis and increased muscle protein breakdown (282, 283). Furthermore, the reduced number and function of the neuromuscular junction may compromise activity intensity (284). Therefore, it is possible that the low accumulation of intensity for given time periods observed is caused by a combined perpetual cycle of functional disability/decline and inactivity. Further evaluation of how physical activity relates to measures of physical frailty would facilitate better understanding of this complex relationship.

4.4.6 Aim 3: Continuous bouts of activity for a given time period

On average, participants with ESLD did not maintain MIPA levels (>100m*g*) beyond one minute, whereas the HC group maintained 30 minutes of continuous MIPA (**Figure 4.5**). This lack of sustained MIPA in the ESLD group indicates reduced utilisation of the oxidative phosphorylation energy system and a potential decline in aerobic function in patients with ESLD. Reduced aerobic function has been found in patients with ESLD (160, 285, 286) and is associated with LT waiting list mortality (160), sepsis (287), hospitalisation post-LT (160) and 90-day and 1-year survival post-LT (160, 161). These findings were supported by the results in Chapter 2 of this thesis which showed that the Duke Activity Status Index (DASI) (a measure of aerobic capacity) was associated with overall and waiting list mortality in patients assessed and/or listed for a LT. Factors that may contribute to this decline in aerobic capacity include: reduced mitochondrial density and function, breathlessness and/or fear of exertion.

As part of the wider evaluation of sarcopenia in liver disease study (described in Chapter 3), Allen et al (2022) investigated mitochondrial function in a subset group (n=4 for each group) of the same HC and participants with ESLD (288). Myotubes were treated with participant serum and it was found that those in the ESLD group had significantly reduced ATP production, mitophagy and mitochondrial reserve than the HC. Furthermore, overall reduced mitochondrial respiration was observed. One of the theoretical causes for this was the significantly higher ammonia levels found in the ESLD group versus the HC. Hyperammonemia, commonly found in patients with ESLD, causes impairment of the electron transport chain via complex I dysfunction, reduced NAD+/NADH ratio and reduced adenosine triphosphate (ATP) content, resulting in oxidative damage (289). Furthermore, it promotes autophagy of skeletal muscle cells and inhibits protein synthesis in skeletal muscle through

stimulation of myostatin (290). Although ammonia was not measured for the HC within this study, the ESLD group had elevated ammonia levels (**Table 4.1**), which may be contributing to their lack of sustained MIPA. These findings combined indicate a need to investigate the role of mitochondria and aerobic capacity in patients with ESLD.

A systematic review conducted by Peng et al. (2019) highlighted that between 20 and 88% of patients with ESLD experience breathlessness (291-295). These findings were comparable to other advanced conditions such as cancer, chronic obstructive pulmonary disorder, chronic heart failure and end-stage renal disease, yet these symptoms are often overlooked in the care of patients with ESLD (296-298). In the study that reported 88% of patients with breathlessness it was found that tidal volume/duration of inspiration (V_T/TI) were increased indicating hyperinflation in 40 patients with ESLD (292). This increase was more pronounced in those with ascites, along with a significant reduction in all respiratory muscle outcomes (292). This is not surprising considering the hampering load of ascites on the thoracic cage, which creates a restrictive lung disease pattern with reduction in both forced expiratory flow (FEV1) and forced vital capacity (FVC), yet preserved FEV1/FVC ratio. This presents as hyperventilation in these patients whereby breathing is rapid and shallow with limited tolerance for apnoea. Therefore, participating in MIPA may precipitate these symptoms in patients with ascites, leading the patient to terminate their activity.

A recent study investigating participation in daily activities in patients with COPD showed that breathlessness was the most common barrier to participation (299). 44% of our cohort presented with refractory ascites, however, throughout the study no guidance was given on participation in physical activity and/or breathlessness management. That, combined with

the alteration in respiratory mechanics and muscle function, indicates a need to provide more support, in the form of breathlessness management, to all patients with ESLD, but in particular those with refractory ascites.

4.4.7 Limitations

The most obvious initial limitation of this study is that it involves a relatively small sample size (n=43), with the majority (55%) of the cohort presenting with ArLD. Furthermore, patients were recruited from a single UK centre and therefore results cannot be generalised to all liver disease types, other centres and those outside of the UK. Additionally, whilst the study provides a good overview of general physical activity profiles, it does not provide information on whether these profiles differ between disease cohorts or are influenced by demographic (i.e. age, sex), clinical (UKELD, ascites, hepatic encephalopathy), or physical frailty status (muscle mass, strength, function). Further research investigating factors associated with physical activity will help guide interventions towards targeted populations.

4.4.8 Contribution to the field

This study is one of only three studies to objectively investigate physical activity patterns in patients with ESLD. Notably, it is the first study to complete an in depth analysis of physical activity intensity modelling in patients with ESLD, whereby the 24-hour profile of physical activity can be quantified. These metrics capture more information than MVPA, which only focuses on a small proportion of the 24-hour day. Moreover, this level of analysis provides a better understanding of the intricacies of physical activity patterns in patients with ESLD and provides a template for future studies to enable comprehensive comparison of physical activity profiles between cohorts.

The recognition of the importance of physical frailty in those listed for liver transplantation has gained significant attraction in recent years, with a move towards establishing Enhanced Recovery After Surgery (ERAS) programmes for those undergoing a LT (300). ERAS is an evidence based multimodal, program of care which is designed to minimise the response to surgical stress (301, 302). Considering physical frailty contributes to many of the complications that ERAS aims to minimise (i.e. hospitalisation, infections, mortality), there is a need to reverse such frailty with the use of interventions such as physical activity programmes. The results from this study will help guide future physical activity intervention design, as well as aid the conveyance of patient friendly physical activity messages, which could be used in the delivery of ERAS programmes for those with ESLD.

Finally, the LT waiting period is a time of great turbulence and uncertainty for patients which has a significant impact on their lifestyle and quality of life (303). Often, healthcare professionals convey messages of change of clinical status that patients have little control over i.e. changes to blood markers, progression of disease. The investigation of physical activity patterns detailed in this study provides a tangible marker for patients that they can directly control which, if delivered effectively, may improve clinical outcomes.

4.5 Conclusion

In summary, UK-based patients with ESLD are significantly less active and have higher sedentary times than HC. Specifically, the total volume of activity intensity and the proportion of the 24-hour period spent at higher intensities is significantly less than HC. Moreover, patients with ESLD accumulated less intensity for given time periods and did not sustain MIPA

beyond one-minute, raising questions regarding the aerobic capacity influence on physical activity patterns. These results provide an in-depth analysis of the intricacies of daily physical activity patterns in those with ESLD. Furthermore, the model of physical activity pattern analysis can be used to form the basis for further investigations such as comparisons of gender, age, disease types, clinical presentation, other measures of physical frailty, and prediction of clinical outcomes.

CHAPTER 5:
ELECTRONIC REMOTE-MONITORING OF PHYSICAL ACTIVITY IN THE CONTEXT OF PHYSICAL FRAILTY MANAGEMENT IN END-STAGE LIVER DISEASE: A CASE CONTROL, OBSERVATIONAL STUDY

5.1 Introduction

Physical inactivity has been widely explored in multiple chronic disease populations (304, 305) and is associated with reduced muscle strength, physical function, greater all-cause mortality and higher risk for diseases such as cardiovascular disease, diabetes and cancer (306). Within the field of end-stage liver disease (ESLD), physical inactivity is associated with overall and liver-related mortality as well as liver transplant (LT) waiting list mortality (272, 273).

Exercise is a subset of physical activity (any bodily movement, produced by skeletal muscle, that requires energy expenditure) that is planned, structured and repetitive with the aim to improve or maintain physical fitness (177). There are two main modes of delivery; aerobic and resistance exercise training. Aerobic exercise training involves repetitional use of large muscle groups which results in improvements in cardiovascular (enhanced cardiac output, improved oxygen uptake) and musculoskeletal (mitochondria biogenesis, muscle hypertrophy) health (307). Resistance exercise training involves exercises performed against a progressive resistance and is used to elicit improved muscle strength, endurance and power (308). Resistance exercise training promotes earl neuromuscular adaptation and, with regular training, stimulation of myofibril, sarcoplasm and connective tissue hypertrophy through the promotion of protein biosynthesis, enhanced endocrinology activity and activation of satellite cells (309).

To mitigate poor clinical outcomes in ESLD, significant attention has been given, with varying success, to physical activity interventions to improve physical frailty (185, 206, 310). In a survey of 165 American Hepatologists 87% recognised, and frequently educated patients on,

the importance of physical activity with many providing specific physical activity recommendations to patients (311). Yet, there remains a lack of standardised physical activity guidelines from the prominent professional associations, American Association for the study of liver disease (AASLD) and European Association for the study of liver disease (EASL). This is likely due to the inconsistencies in physical activity/exercise trials in ESLD to date. Generally, physical activity interventions have shown improvements in aerobic capacity, muscle strength, muscle function and physical performance (178, 183, 185, 187, 194). Nevertheless, studies are often limited by small numbers, heterogeneity of disease aetiologies, predominantly involve patients with low disease severity (i.e. low Model for ESLD (MELD)/United Kingdom MELD (UKELD)/Child-Pugh Score (CPS) A/B) and are observational in nature (206), thus, little is known on who is likely to gain the most benefit. Additionally, heterogeneity between physical activity type (i.e. aerobic, resistance, moderate/high intensity), setting (i.e. hospital versus home-based) and reported adherence (6-100%) hinders the ability of clinicians to provide evidence-based physical activity advice for patients with ESLD. Likewise, there is little in the way of guidance to inform future study protocol interventions.

Consequently, physical activity interventions and clinical advice to date rely on generic recommendations from the World Health Organisation (WHO) and/or American College of Sports Medicine (ACSM) (213, 312). Whilst little harm is likely to arise from using such recommendations, it has been found that different populations benefit from targeting different types of physical intervention (257, 280, 313). For example, Dawkins and colleagues found that those with chronic disease who engaged in a greater volume of activity regardless of intensity had lower cardiometabolic risk, whereas in healthy controls, both intensity and

volume of daily physical activity were higher in those with lower cardiometabolic risk (280). Therefore, without better understanding of physical activity profiles and their impact on physical frailty in ESLD, targeted treatment for these patients will continue to be inadequate.

Hence, this study aimed to determine which clinical and physical frailty measures are associated with physical inactivity and to investigate the relative contribution of the overall volume and intensity of physical activity on physical frailty in ESLD. The following aims will be addressed:

Aim 1: Compare the volume and intensity of physical activity across a healthy control (HC) group and different ESLD aetiologies.

Aim 2: Determine associations between the volume/intensity of physical activity and clinical features/disease severity in patients with ESLD.

Aim 3: Determine associations between the volume/intensity of physical activity and measures of physical frailty in patients with ESLD.

Aim 4: Determine differences in the physical activity profile across quartiles of physical frailty severity in patients with ESLD.

5.2 Methods

5.2.1 Recruitment and assessment of physical frailty and physical activity

Participants with ESLD and HC were recruited as per section 3.3. Physical frailty and remotely-monitored physical activity data were collected and analysed as per the methods laid out in section 3.5. Physical frailty measures included: Liver Frailty Index (LFI), chair stands, dominant hand grip, gait speed, quadriceps strength (assessed by isokinetic peak torque), ultrasound

guided vastus lateralis (VL) thickness and quadriceps anatomical cross-sectional area (ACSA).

For the purpose of this chapter, the physical activity metrics analysed were;

a) volume of physical activity, i.e. average acceleration (m*g*)
b) intensity distribution of physical activity, i.e. intensity gradient
c) intensity of the most active accumulated 2, 5, 10, 15, 30, 60 and 120 minutes of the day and the
d) intensity of the most active continuous 1, 2, 5, 10, 15, 30, and 60 minutes of the day

All metrics were calculated for each valid day and consequently averaged across all valid days.

5.2.2 Data analysis and statistical approach

Data were analysed using IBM SPSS statistics software (version 28.0) and GraphPad Prism (version 9) and the level of significance was set at $p<0.05$ for all statistical tests. All continuous data are presented as median (interquartile range [IQR]), while categorical data is presented as a number (n) and percentage (%).

5.2.2.1 Aim 1

Comparisons between the disease aetiologies, namely alcohol related liver disease (ArLD), non-alcohol related fatty liver disease (NAFLD) and autoimmune liver disease (AID), and HC were made for both average acceleration (volume of physical activity) and intensity gradient. Differences between two groups (i.e. ESLD and HC) was analysed using an unpaired two-tailed t-test (parametric) or a Mann Whitney U test (non-parametric). For between group analysis, parametric and non-parametric data were analysed by means of a One way ANOVA and Kruskal-Wallis test, respectively. A Pearson's correlation coefficient was used to investigate the relationship between average acceleration and intensity gradient of physical activity.

5.2.2.2 Aim 2

Single linear regression was completed to assess whether the demographic and clinical variables (age, sex, BMI, UKELD, refractory ascites and refractory hepatic encephalopathy) were predictors of either of the dependent variables: a) average acceleration and b) intensity gradient. Dummy variables were created for the categorical variables; sex, refractory ascites and refractory hepatic encephalopathy. Then the following assumptions were tested prior to proceeding (314): linear relationship, by visualisation of a scatter plot; independence of residuals, as assessed by a Durbin-Watson statistic; homoscedasticity of residuals, as assessed by visual inspection of a plot of standardised residuals versus standardised predicted values; significant outliers, assessed by case wise diagnostics (standardised residual set to >±3), and observed approximate normal distribution of the residuals.

A multiple regression analysis was completed to assess whether the demographic and clinical variables (age, sex, BMI, UKELD, refractory ascites and refractory hepatic encephalopathy) were associated with average acceleration and intensity gradient independently of each other. The assumptions: independence of residuals, linear relationship, homoscedasticity of residuals, and approximate normal distribution of the residuals were tested as described above. Multicollinearity was checked by calculating the variance inflation factor (VIF) (values >5 indicated unreliable estimates of the predictors). Finally, testing of leverage and influential points, were completed prior to multiple regression analysis. Values >3x average leverage (calculated as per Box 1) were deemed to have undue leverage (315), and a Cook's distance >1 was cause for further investigation (316).

Box 1: Average leverage for this study

Average leverage = 3(k+1/n) = 3(9+1/43) = 0.70

Note: k=nº of predictors, n=nº of participants

5.2.2.3 Aim 3

Regression analyses were also repeated as outlined above to predict average acceleration and intensity gradient from the physical frailty-related independent variables; LFI, chair stands, dominant hand grip, gait speed, quadriceps strength, ultrasound guided VL muscle thickness and quadriceps ACSA.

5.2.2.4 Aim 4

LFI quartiles were created based on the descriptive data collated on the 307 patients listed for liver transplantation in Chapter 2. Comparison of average acceleration and intensity gradient across LFI quartiles were made using the non-parametric between group Kruskal-Wallis test. To illustrate the physical activity pattern associated with each LFI quartile, the mean (standard error of the mean [SEM]) of the MX metrics (i.e. M2-M480) for both accumulation, and continuous bouts of, most active minutes for each LFI quartile were calculated and displayed on radar plots. Radar plots were generated in Rstudio (version 2.0) using the github code: https://github.com/Maylor8/RadarPlotGenerator (229). Standardised MX metrics were also calculated for each MX metric relative to the mean and standard deviation (SD) of the whole sample (i.e. ESLD) and consequently plotted. Dashed circles and walking values are also plotted for reference values in an identical format to Chapter 4 for visualisation of physical activity profiling.

5.3 Results

5.3.1 Demographics

43 participants with ESLD, median age 56.0 years (Interquartile range (IQR) 50.0-60.0), 65% male (n=28) with the disease aetiologies ArLD (n=23), NAFLD (n=5), AID (n=13), or other (n=2) and a median UKELD 52.0 (IQR 50.0-55.0), and 17 age/sex matched healthy controls (median age 47.0 years (IQR 33.5-58.8), 56% (n=10) male) were included in the analysis. 44 and 21% of individuals with ESLD had refractory ascites (diuretic resistant, requiring paracentesis) and refractory hepatic encephalopathy (grade I/II west-haven despite rifaximin/lactulose), respectively. There was no significant difference in demographics or clinical characteristics between the three disease aetiology groups. However, BMI was significantly lower in the HC group when compared to those with ESLD (24.4 vs 29.2kg/m^2, $p<0.01$). These data are taken from the main demographic table presented in Chapter 4 (**Table 4.1**) and are summarised with the inclusion of a disease aetiology breakdown in **Table 5.1**.

The ESLD group were significantly frailer in all measures of physical frailty when compared to the HC group. However, there were no significant differences in physical frailty markers between the disease aetiology groups of ArLD, NAFLD and AID (**Table 5.2**).

Table 5.1 Demographics and clinical characteristics of participants with ESLD and according to disease aetiology

Clinical Characteristic	ESLD (n=43)	HC (n=17)	p-value^	ArLD (n=23)	NAFLD (n=5)	AID (n=13)	p-value^^
Age	56.0 (50.0-60.0)	47.0 (35.5-58.8)	0.08	54.0 (52.0-59.0)	54.0 (45.5-59.5)	57.0 (37.5-66.5)	0.60
Sex (male)	28 (65%)	10 (56%)	0.65	16 (70%)	2 (40%)	8 (62%)	0.43
BMI(kg/m^2)	29.2 (24.2-34.2)	24.4 (22.1-27.7)	**<0.01**	31.0 (26.0-34.7))	30.3 (24.7-39.9)	26.6 (22.4-31.2)	0.27
UKELD	52.0 (50.0-55.0)	-	-	54.0 (50.0-56.0)	50.0 (49.0-54.0)	52.0 (51.5-53.0)	0.24
Refractory ascites	19 (44%)	-	-	15 (65%)	2 (40%)	0 (0%)	**<0.001**
Refractory HE	9 (21%)	-	-	7 (30%)	1 (20%)	1 (7%)	0.31

Note: Data expressed as median and interquartile range. Significance is highlighted in bold.
^p-value represents comparison between ESLD and HC groups using unpaired t-test or Mann Whitney U test for continuous data and chi-squared test for categorical data.
^^ p-value represents analysis of overall difference between disease aetiologies using One way ANOVA or Kruskal-Wallis test for continuous data and Fisher's exact test for categorical data.
Abbreviations: AID = Autoimmune liver disease, ArLD = alcohol-related liver disease, BMI = body mass index, ESLD = end-stage liver disease, HC = healthy controls, HE = hepatic encephalopathy, kg/m^2 = kilograms per metre squared, NAFLD = non-alcohol-related liver disease, UKELD = United

Table 5.2 Physical frailty outcomes in participants with ESLD and according to disease aetiology

Physical frailty measures	Total (n=43)	HC	p-value^	ArLD (n=23)	NAFLD (n=5)	AID (n=13)	p-value^^
LFI	3.59 (3.3-3.6)	2.9 (2.4-3.2)	**<0.01**	3.6 (3.3-4.0)	3.9 (3.6-3.9)	3.5 (3.1-3.7)	0.28
Chair stand time (secs)	10.4 (9.0-12.0)	7.7 (5.8-10.4)	**<0.01**	10.4 (8.4-14.5)	10.9 (10.1-12.3)	9.8 (8.3-11.1)	0.48
Hand grip (kg)	31.4 (24.7-38.5)	37.9 (28.8-47.0)	**0.02**	32.9 (24.8-39.9)	21.3 (18.4-30.1)	31.6 (24.0-40.8)	0.10
Gait speed (m/s)	0.9 (0.8-1.1)	1.2 (1.1-1.3)	**<0.01**	1.0 (0.8-1.2)	1.1 (0.8-1.2)	0.9 (0.8-1.1)	0.50
Quad strength (peak torque, Nm)	104.4 (74.9-133.0)	137.8 (101.1-191.5)	**<0.01**	95.9 (69.4-123.0)	113.8 (96.5-134.3)	113.5 (79.0-189.0)	0.20
VL thickness (cm)	2.1 (1.9-2.3)	2.40 (1.9-2.8)	**0.01**	2.1 (1.9-2.3)	2.0 (1.5-2.3)	2.0 (1.9-2.3)	0.73
Quad ACSA (cm^2)	50.1 (41.3-55.1)	63.2 (47.3-99.7)	**<0.01**	51.2 (41.8-54.0)	42.9 (32.7-65.5)	50.1 (39.8-61.4)	0.90

Note: Data expressed as median and interquartile range. Significance highlighted in bold.
^p-value represents comparison between ESLD and HC groups using unpaired t-test or Mann Whitney U test.
^^ p-value represents analysis of overall difference between disease aetiologies using One way ANOVA or Kruskal-Wallis test.
Abbreviations: AID = Autoimmune liver disease, ArLD = alcohol-related liver disease, ACSA = anatomical cross-sectional area, cm = centimetres, HC = healthy controls, kg = kilogram, LFI = liver frailty index, m/s = metres per second, NAFLD = non-alcohol-related liver disease, Nm = newton metre, Quad = quadriceps, VL = vastus lateralis

5.3.2 Aim 1: Comparison of the volume and intensity of physical activity across the HC group and liver disease aetiologies

Participants with ArLD had significantly lower average acceleration than those with AID (mean (Standard deviation [SD]) 13.4(4.6) vs 23.1(7.1) milli-gravitational units (m*g*), p<0.01) and HC (13.4(4.6) vs 29.2(8.9)m*g* , p<0.01) (**Figure 5.1A**). However, there was no significant difference in average acceleration when comparing those with ArLD and NAFLD. Nevertheless, there was a general trend from higher to lower average acceleration, with HC accumulating the highest average daily acceleration and the ArLD group the least (**Figure 5.1A**).

Individuals with ArLD had the lowest recorded intensity gradient and therefore varied the intensity of their physical activity the least, spending proportionately more of their time at low intensity (**Figure 5.1B**). Both the ArLD and AID groups had significantly lower intensity gradients than HC (-2.9 vs -2.4, p<0.01 and -2.7 vs -2.4, p<0.01, respectively). No significant difference in intensity gradient between those with NAFLD and HC was seen (**Figure 5.1B**).

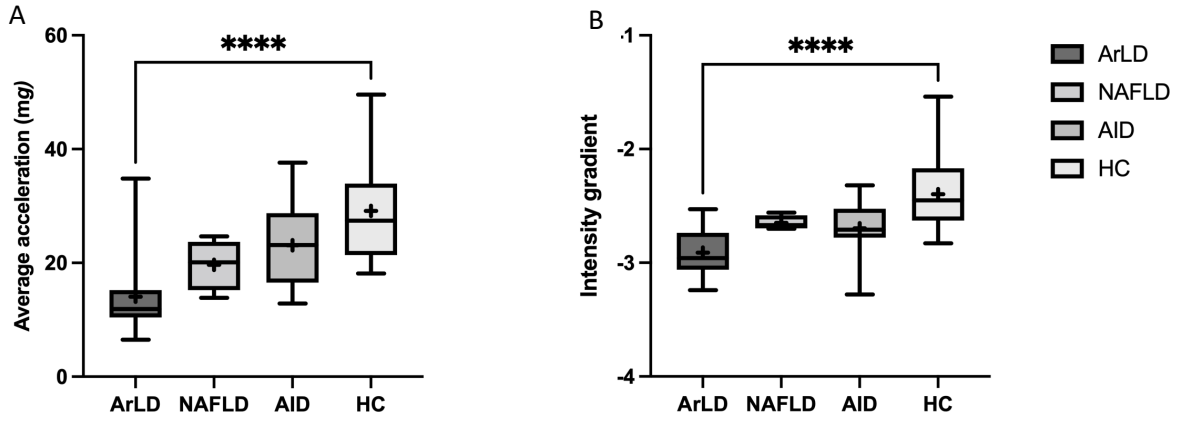


Figure 5.1 Graphs comparing: (A) total volume of activity (average acceleration) and (B) the distribution of intensity participation (intensity gradient) between the groups ArLD, NAFLD AID and HC

Note: The higher the gradient the greater the distribution of activity over inactive, light, moderate and vigorous intensities. As such, ArLD spent proportionately more of their time at low intensity.
Data are expressed as median (central horizontal line), 25th and 75th percentile (box) and the minimum and maximum (horizontal line).
+mean
**** p<0.0001

5.3.3 Aim 2: The association between demographic and clinical factors and the volume and intensity of physical activity in ESLD

There was a moderate-strong (r=0.70) correlation between average acceleration and intensity gradient, confirming that the two measures provided complimentary, yet independent information (**Figure 5.2**).

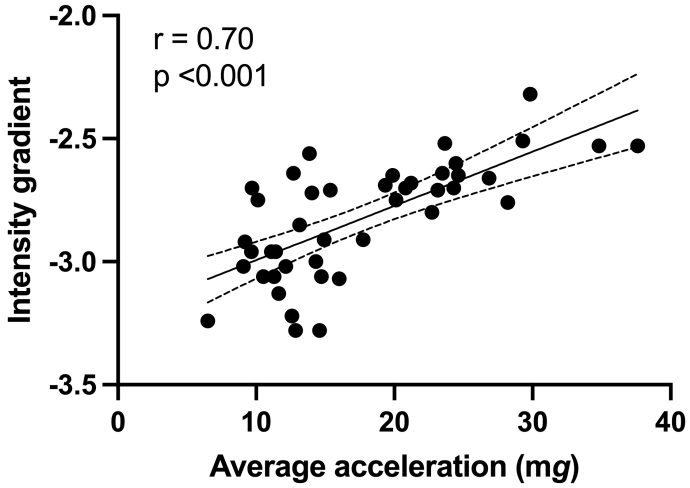


Figure 5.2 Correlation between average acceleration (total volume) and intensity gradient

Single regression analysis, where all assumptions were met, demonstrated that only the presence of refractory ascites was significantly associated with lower average acceleration (B=-7.18, 95% CI -11.27 to -3.08, p<0.01), and only older age was significantly associated with lower intensity gradient (B=-0.01, 95% CI -0.02 to -0.001, p=0.03) (**Table 5.3**). The multiple regression model statistically predicted average acceleration (F=4.05, p 0.003, adj. R^2=0.30), where older age and the presence of refractory ascites were significantly associated with lower average acceleration (B=-0.24, 95% CI -0.44 to -0.03, p=0.02 [age], B=-7.46, 95% CI -11.62 to -3.32, p<0.01 [refractory ascites]). Older age and the presence of refractory ascites were also independent predictors of a lower intensity gradient (B=-0.01, 95% CI -0.16 to -0.002, p=0.02 [age], B=-0.15, 95% CI -0.29 to 0.00, p=0.04 [refractory ascites]), however, this multiple regression model was weaker and did not statistically predict intensity gradient (F=2.12, p=0.08, adj. R^2=0.14) (**Table 5.3**). Of note, sex, BMI, UKELD and refractory hepatic encephalopathy did not significant predict average acceleration or intensity gradient in either single or multiple regression.

Table 5.3 Single and multiple regression analysing demographic and clinical predictors of physical activity

Variable		Single linear regression			Multiple linear regression		
		B	95% CI	p-value	B	95% CI	p-value
Age	Average acceleration	-0.214	-0.44 to 0.01	0.06	**-0.24**	**-0.44 to -0.03**	**0.02**
	Intensity gradient	**-0.01**	**-0.02 to -0.001**	**0.03**	**-0.001**	**-0.16 to -0.002**	**0.02**
Sex	Average acceleration	-0.148	-5.02 to 4.73	0.95	2.63	-2.04 to 7.30	0.26
	Intensity gradient	0.02	-0.14 to 0.17	0.84	0.08	-0.09 to 0.24	0.35
BMI	Average acceleration	-0.22	-0.57 to 0.13	0.21	-0.13	-0.46 to 0.20	0.44
	Intensity gradient	5.80	-0.01 to 0.11	0.99	0.004	-0.01 to 0.02	0.52
UKELD	Average acceleration	-0.53	-1.23 to 0.17	0.13	-0.34	-1.00 to 0.33	0.31
	Intensity gradient	-0.01	-0.03 to 0.01	0.43	-0.010	-0.03 to 0.01	0.39
Refractory HE	Average acceleration	-2.27	-7.93 to 3.40	0.42	-1.86	-7.60 to 3.88	0.52
	Intensity gradient	-0.07	-0.25 to 0.11	0.44	-0.08	-0.28 to 0.12	0.42
Refractory ascites	Average acceleration	**-7.18**	**-11.27 to -3.08**	**<0.01**	**-7.46**	**-11.62 to -3.32**	**<0.01**
	Intensity gradient	-0.14	-0.28 to 0.003	0.05	**-0.15**	**-0.29 to 0.00**	**0.04**

Note: Significant predictors highlighted in bold.
Abbreviations: B = beta coefficient, BMI = body mass index, CI = confidence interval, HE = hepatic encephalopathy, UKELD = United Kingdom model for end-stage liver disease

5.3.4 Aim 3: The association between measures of physical frailty and the volume and intensity of physical activity

Single regression analysis was completed to determine the predictive value of measures of physical frailty on physical activity profiling (**Table 5.4**). All assumptions were met as part of the analysis. The single regression model showed that LFI and quadriceps extensor strength were significantly associated with both lower average acceleration (B=-4.68, 95% CI -8.74 to 0.614, p=0.03 [LFI], B=0.11, 95% CI 0.04 to 0.18, p<0.01 [quad strength]) and lower intensity gradient (B=-0.21, 95% CI -0.33 to -0.09, p<0.01 [LFI], B=0.004, 95% CI 0.002 to 0.006, p<0.01 [quad strength]). However, there was no statistically significant relationship between average acceleration and intensity gradient and the physical frailty variables: chair stands, hand grip strength, VL muscle thickness and quadriceps ACSA (**Table 5.4**).

Both LFI and extensor quadriceps strength were predictors of both physical activity in the single regression analysis. However, as LFI is easier-to-use and more assessable in the clinical setting, I elected to utilise LFI in the multiple regression analysis rather than extensor quadricep strength (isokinetic dynamometry). Multiple regression analysis was completed to assess the predictive ability of the LFI on physical activity variables, when controlled for age and ascites, as these were previously demonstrated to be the strongest predictors of physical activity (section 5.3.3). The multiple regression models statistically predicted both average acceleration (F=7.56, p<0.01, adj. R^2=0.33) and intensity gradient (F=10.38, p<0.001, adj. R^2=0.32), where higher LFI was associated with lower average acceleration and lower intensity gradient when controlled for age and ascites (

Table 5.5).

Table 5.4 Single linear regression analysing measures of physical frailty as predictors of physical activity

Variable		Single regression		
		B	95% CI	p-value
LFI	Average acceleration.	**-4.68**	**-8.74 to 0.614**	**0.03**
	Intensity gradient	**-0.21**	**-0.33 to -0.09**	**<0.01**
Chair stands (x5)	Average acceleration.	-0.12	-0.60 to 0.36	0.61
	Intensity gradient	0.01	-0.02 to 0.01	0.45
Hand Grip	Average acceleration.	0.10	-0.19 to 0.39	0.48
	Intensity gradient	0.01	-0.003 to 0.01	0.22
Gait speed	Average acceleration.	1.53	-7.91 to 10.98	0.75
	Intensity gradient	-0.07	-0.37 to 0.23	0.62
Quad strength	Average acceleration.	**0.11**	**0.04 to 0.18**	**<0.01**
	Intensity gradient	**0.004**	**0.002 to 0.006**	**<0.01**
VL thickness	Average acceleration.	-1.29	-7.73 to 5.15	0.69
	Intensity gradient	0.01	-0.19 to 0.22	0.90
Quad ACSA	Average acceleration.	0.02	-0.48 to 0.26	0.89
	Intensity gradient	0.003	-0.01 to 0.01	0.78

Note: Significant predictors highlighted in bold.
Average acceleration: single regression analysis, analysing predictive ability of independent variable on average acceleration
Intensity gradient: single regression analysis, analysing predictive ability of independent variable on intensity gradient
Abbreviations: ACSA = anatomical cross sectional area, B = Beta coefficient, CI = confidence interval, LFI = liver frailty index, Quad = quadriceps, VL = vastus lateralis

Table 5.5 Multiple regression analysing measures of physical frailty as predictors of physical activity

Variable		Multiple regression					
		B	95% CI	p-value	Adj.R^2	F	p-value
LFI	Average acceleration	-3.65	-7.21 to -0.08	0.045	0.33	7.56	**<0.01**
	Intensity gradient	-0.21	-0.33 to -0.09	<0.001	0.32	10.38	**<0.001**

Note: Significant predictors highlighted in bold.
Average acceleration: multiple regression analysing predictive ability of LFI when controlled for age and ascites
Intensity gradient: multiple regression analysing predictive ability of LFI when controlled for age (ascites excluded from regression due to high multicollinearity)
Abbreviations: Adj. = adjusted, B = Beta coefficient, CI = confidence interval, F = f-statistics (mean sum of squares regression/mean sum of squares error), LFI = liver frailty index

5.3.5 Aim 4: Differences in the physical activity profile across quartiles of the severity of physical frailty

Average acceleration and intensity gradient were compared across four quartiles of the LFI (1^{st} = <3.37, 2^{nd} = 3.38-3.78, 3^{rd} = 3.79-4.28, 4^{th} = >4.28; with the 4^{th} being the most severe). There was no significant overall effect (p=0.08) or between group difference in average acceleration (**Figure 5.3A**). However, there was a significant overall effect (p=0.002), and a significant between group difference in average intensity gradient for those who were least frail (1^{st} quartile, red bar) and most frail (4^{th} quartile, purple bar) (mean(SD) -2.7(0.2) vs -3.1(0.1), p=0.004) (**Figure 5.3B**), as well as between those in the 2^{nd} and 4^{th} quartile of physical frailty (-2.8(0.2) vs -3.1(0.1), p=0.02). Yet, there was no significant difference between 1^{st} and 2^{nd}, 1^{st} and 3^{rd}, 2^{nd} and 3^{rd}, or 3^{rd} and 4^{th} LFI quartiles (**Figure 5.3B**).

Despite non-significance, it is evident from the downward trend seen in Figure 5.3 A and B that the more frail a patient is (i.e. higher LFI score), the lower the average acceleration and intensity gradient, respectively. The MX metrics for all groups are displayed within **Figure 5.3** for acceleration values (5.3C) and standardised differences relative to the mean and standard deviation (5.3D) of the whole ESLD group. Individuals who were the least physically frail (1^{st} quartile, red line), i.e. defined as robust, accumulated 60 minutes of activity at an intensity indicative of slow walking compared to 30 minutes in the middle two quartiles (green and blue lines) and only 10 minutes for those who were most physically frail (4^{th} quartile, purple line). Notably, even the most robust group accumulated just 5 minutes at an intensity indicative of a brisk walk, highlighting the low level of physical activity in this patient population (**Figure 5.3C**). Differences in intensity between those who were robust and most

physically frail were approximately 1.0SD across the physical activity profile, irrespective of MX metric (**Figure 5.3D**).

The intensity of the most active continuous 1 to 60 minutes, by frailty quartile, is shown in **Figure 5.4**. There was an overall significant difference in average acceleration for the most active continuous time periods between LFI quartiles for one, two and five minute time periods ($p<0.05$). There was a significant difference between the 1st and 4th LFI quartile for five minutes, yet no between group difference for any other time period (1-2 or 10-60 minutes). However, those who were least frail, i.e. robust, participated in continuous vigorous activity (400m*g*) for one minute, but did not sustain activity indicative of a slow walk beyond two minutes. Whilst the middle two LFI quartiles reached a brisk walk for one minute, this was not maintained beyond this and, like the most frail group, did not maintain a continuous intensity indicative of a slow walk beyond one minute (**Figure 5.4**).

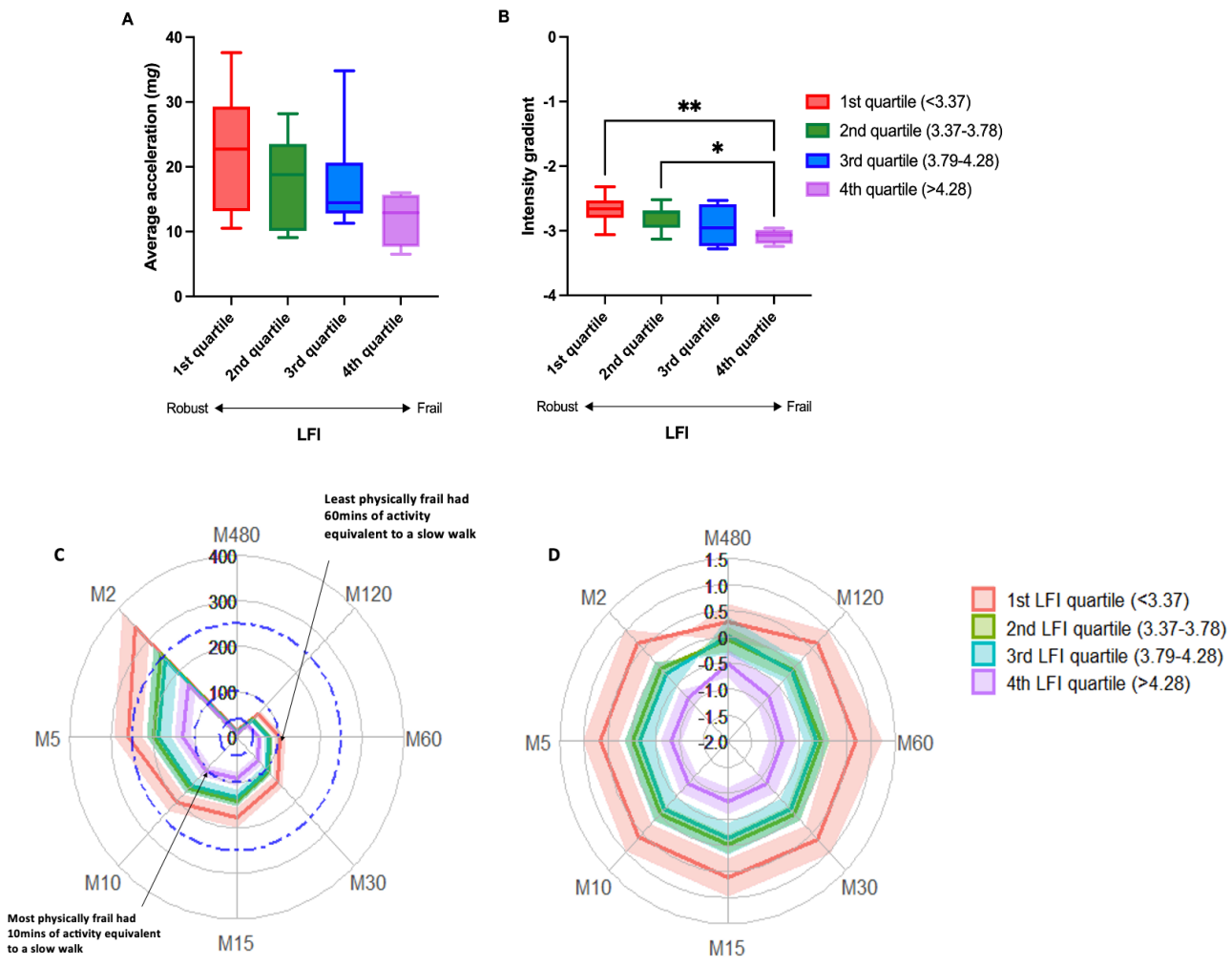


Figure 5.3 Translation of the effect of average acceleration and intensity gradient on physical frailty, as measured by the Liver Frailty Index (LFI)

A: the relationship between average acceleration and the 1st (<3.37), 2nd (3.38-3.77), 3rd (3.78-4.28) and 4th (>4.28) quartiles of the LFI (quartiles based on cohort of 307 patients in Chapter 2).

B: the relationship between intensity gradient and the 1st, 2nd, 3rd, and 4th quartile of the LFI.

C: illustration of the physical activity profile for raw MX metrics.

D: standardised MX metrics associated with the 1st, 2nd, 3rd, and 4th LFI quartiles.

Note: The colour of the lines in radar plots C and D correspond with the colour of the bars in graphs A and B.

Data in A and B are expressed as median (central horizontal line), 25th and 75th percentile (box) and the minimum and maximum (horizontal line).

Blue dashed lines = 100m*g* (activity indicative of a slow walk) and 250m*g* (activity indicative of a brisk walk

** p<0.005

*p<0.05

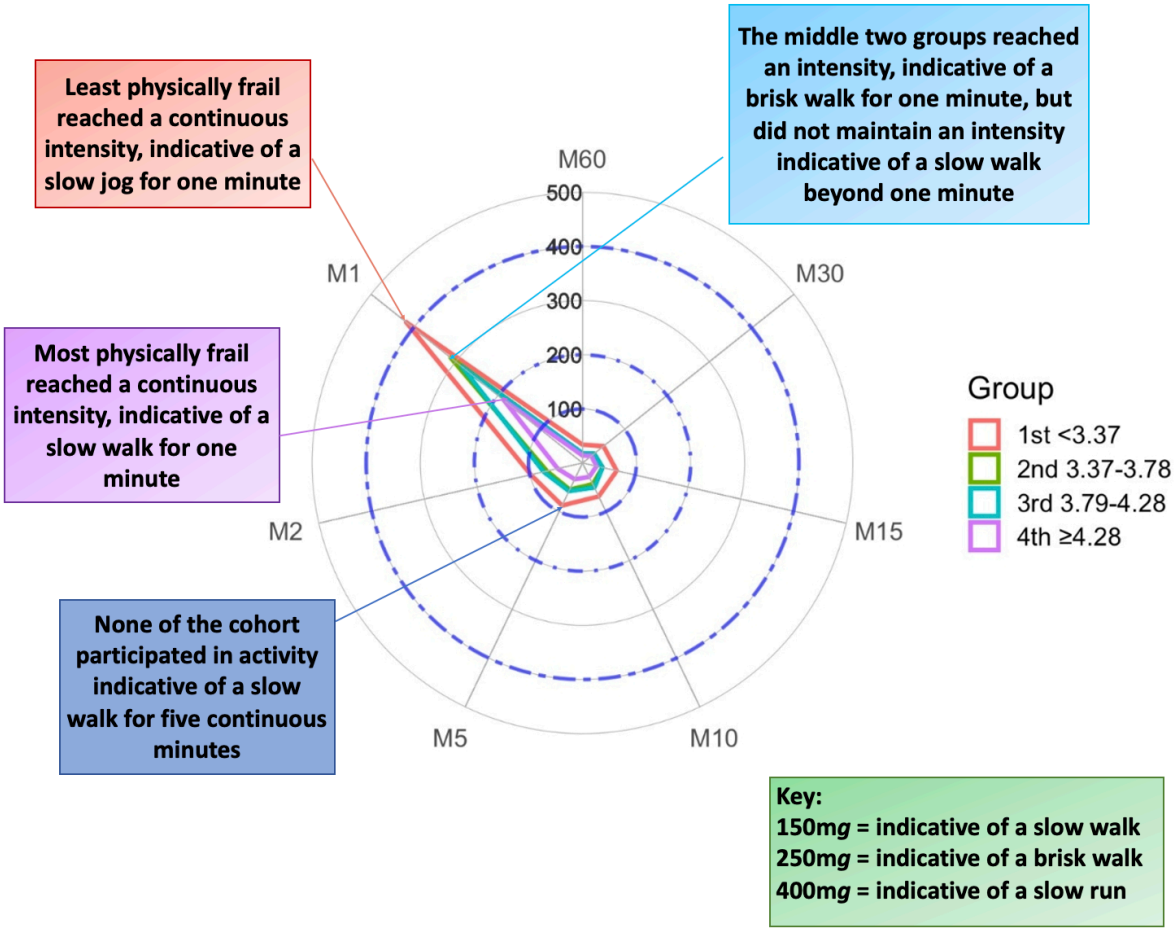


Figure 5.4 Radar plot illustrating metrics for average acceleration for the most active continuous 60mins (M60), 30 mins (M30), 15mins (M15), 10mins (M10), 5mins (M5), 2 mins (M2) and 1 min (M1) for LFI quartiles

5.4 Discussion

The results of Chapter 4, along with previous studies, have suggested that those with ESLD are physically inactive (273, 276). However, predictors (i.e. demographic, disease aetiology, clinical characteristics) of physical inactivity in ESLD are yet to be understood. Furthermore, whilst there is an established general link between physical inactivity and physical frailty (317), the exact mechanisms are unclear and there is no understanding of the amount and intensity of activity needed to improve physical frailty in those with ESLD. Therefore, this chapter aimed to understand specific predictors of the volume and distribution of intensity of physical activity and how this, in turn, impacts on physical frailty. This understanding is important to guide the development of physical activity interventions tailored specifically to improving physical frailty in ESLD.

5.4.1 Key Findings

All disease groups of ESLD had lower intensity and volume of activity than HC, but this was only significant for ArLD and AID for intensity and ArLD for volume. Clinically, both age and refractory ascites were independent predictors of both average acceleration and intensity gradient. Whereas, from a physical frailty assessment perspective, the LFI and isokinetic quadriceps extensor strength were the strongest predictors of both average acceleration and intensity gradient. Additionally, the LFI was an independent predictor of both average acceleration and intensity gradient when controlled for age and refractory ascites. Physical activity intensity decreased as frailty increased, with those with lower LFI scores (i.e. least physical frailty) gaining proportionately more physical activity at higher intensities than those with the highest LFI (i.e. the frailest). Physical activity volume also decreased as frailty increased, although this did not reach significance. Overall, all of the participants with ESLD

did not participate in continuous physical activity at a level indicative of a slow walk for more than two minutes. However, those who were least frail maintained vigorous intensity (400m*g*) activity, indicative of slow running for one minute.

5.4.2 Aim 1: Impact of disease aetiology on physical activity

Studies investigating physical activity have thus far focused on ESLD as a whole and not considered differences between disease aetiologies. This study found there was a downward trend from HC, to AID, NAFLD and finally ArLD in terms of overall activity, measured by average acceleration. This trend was replicated for intensity gradient, with ArLD and AID recording significantly lower intensity gradients than HC, but not NAFLD. The latter may be explained by the small sample size of NAFLD (n=5).

Overall, both the total volume and the intensity distribution of physical activity are compromised in patients with ESLD. The fact that individuals with ArLD recorded the lowest average acceleration and intensity gradient may be explained by two key reasons. Firstly, in addition to the cirrhosis induced imbalance in skeletal muscle proteolysis in ArLD (318-321), there is a known added influence of ethanol on muscle health (322-325). Whilst ethanol is primarily metabolised in the brain and liver, it can also be metabolised in skeletal muscle (323, 326, 327). Chronic high doses of ethanol ingestion leads to reduced muscle protein synthesis and subsequent muscle function through impaired mTORC1 signalling, increased myostatin and increased autophagy (328-331). Furthermore, ethanol causes impairment of mitochondrial function leading to the cascade of increased reactive oxidative species, activation of mitophagy and eventual reduction in adenosine tri-phosphate (ATP) production (325). Whilst some of these consequences may have reversed due to the prolonged period of

abstinence within this cohort (median abstinent time 24 months), it is not known if full recovery of the skeletal muscle is ever achieved, making it difficult to know if impaired muscle function, and subsequent reduced physical activity in this group, results from previous exposure to ethanol or its metabolites (330). Transcriptomic analysis of muscle may be useful to address this question as it could reveal signatures associated with alcohol exposure. Secondly, the majority (65%) of participants within the ArLD group had refractory ascites. In our multiple regression analysis, refractory ascites was an independent predictor of both average acceleration and intensity gradient. Thus, ascites may be influencing inactivity in this cohort, rather than specific mechanisms derived from ArLD itself, however further research is needed in this area to confirm this.

5.4.3 Aim 2: Impact of age, gender and disease severity on physical activity

Both age and refractory ascites were independent predictors of low average acceleration and intensity gradient. This is in keeping with other 'age' data in the UK, where sedentary behaviour, or inactivity, has been shown to increase with ageing, with less than 10% of the UK 'older' population (age >65 years) meeting the UK Government physical activity guidelines (332, 333). Higher rates of inactivity seen in our older participants with ESLD may well be accelerating the combined low level chronic inflammation and oxidative stress seen in both older age and ESLD (334, 335). This promotes a vicious cycle of reduced muscle protein synthesis, increased anabolic resistance (336), and reduced satellite cells (337), resulting in both loss of lean muscle mass and muscle strength (338), which can then influence activity levels. In view of these changes and the known positive impact physical activity has not only on physical frailty in ESLD (206, 311, 339), but also in promoting healthy ageing (i.e. preserved

health, physical, social and mental wellness (340)) (338, 341-343), access to physical activity interventions for older patients with ESLD is needed.

Refractory ascites was the only clinical characteristic to predict both average acceleration and intensity gradient. This finding supports the theories outlined in section 4.5.6 whereby the abdominal discomfort and symptoms of breathlessness seen in patients with ascites (291, 344) may hinder participation in physical activity. Additionally, ascites promotes early satiation, which can result in reduced oral intake and calorie deficit, and increased protein loss, which further exacerbates the imbalance of skeletal muscle protein turnover in those with ESLD (345). Therefore, careful attention to improve physical activity engagement, alongside optimal nutritional support and management of ascites (i.e. regular paracentesis and diuretics) is needed to avoid decline in physical frailty for those with refractory ascites.

Interestingly, despite the cognitive deficits surrounding refractory hepatic encephalopathy, it was not a significant predictor of average acceleration or intensity gradient. This is in keeping with other evidence that suggests the impairments of daily function for those with hepatic encephalopathy is not related to daily activities such as shopping and dressing, but more complex activities that require attention, information processing and psychomotor skills (346, 347). However, our data may be limited by recruitment bias, as only outpatient participants, i.e. those with less severe hepatic encephalopathy (grade ≤2) were recruited, who may be less debilitated than those with more severe refractory hepatic encephalopathy.

5.4.4 Aim 3: Relationship of physical frailty and physical activity

Physical frailty variables relating to muscle function and muscle strength (i.e. LFI, peak extensor quadriceps strength) were significant predictors of average acceleration and intensity gradient, whereas measures of muscle mass were not. These findings are in line with the guidance of the European Working Group on Sarcopenia in Older People (EWGSOP) who recognise low muscle strength as a key characteristic of sarcopenia. The EWGSOP recommend identifying patients with a discrepancy in muscle strength before determining any loss of quantity or quality of muscle, followed by a severity assessment in the form of measuring the impact on physical performance (348), which in the case of this study would be physical activity profiling.

The association of lower limb strength with remotely-monitored physical activity found in this study is supported by other non-liver studies. For example, in a cohort of 636 Australian community-dwelling older adults, Foong et al. (2016) found a dose-response relationship between physical activity and lower limb strength, where the greater the intensity of the activity performed, the higher the lower limb strength (349). Similarly, Menant et al. (2017) found that a simple lower limb extensor strength test was just as effective at predicting functional mobility and falls in older patients as measures of muscle mass (350). Whilst both of these studies were conducted in a cohort of older adults, rather than ESLD, in our wider study of evaluating sarcopenia in chronic inflammatory disease (ESCID), we found significant differences in peak extensor quadriceps strength between patients with ESLD and HC, but no difference between magnetic resonance imaging (MRI) informed skeletal muscle index at the 3rd lumbar vertebrae (L3) or mid-arm muscle circumference (MAMC) (351).

Interestingly, a large proportion of the physical frailty literature from North America focuses on sarcopenia (defined in North America as the loss of muscle mass (77)) as a key predictor of outcome in ESLD (154, 198, 352-357). Whilst this is important to acknowledge, the lack of sensitivity of measures of muscle mass in the context of physical activity seen in this study suggests they may not be clinically useful. Furthermore, measures of muscle mass, such as the skeletal muscle index at L3 assessed by computed tomography (CT) and MRI are expensive, time burdensome and, in the case of CT, the patient is exposed to radiation (234). Physical activity is known to have the potential to improve physical frailty in patients with ESLD. Thus, if measures of muscle mass are not able to predict physical activity, then they have limited use in monitoring response to physical activity interventions. Future research studies and clinical services should focus upon measures of muscle strength and function, and not muscle mass, when prescribing and monitoring physical activity interventions.

Whilst peak extensor quadriceps strength was a significant predictor of both average acceleration and intensity gradient in our study, it requires costly equipment which is not widely available within the clinical setting. The more practical, cheaper measure of lower limb strength, chair stands, did not significantly predict physical activity markers in our study. This may be due to the various confounding variables that can influence a timed chair stand test. For example, isokinetic quadriceps strength testing is completed with the patient in a fixed position with quadriceps isolated making the test highly specific. In comparison, chair stands may be influenced by clinical factors such as ascites, or other contributors to physical performance such as balance, coordination and core strength. Nonetheless, when chair stands were combined with hand grip and balance as part of the LFI, significant prediction of physical activity markers were found. The rationale for this result is unclear and further

investigations with larger study population may be needed. Overall though our study adds to the established strength of the LFI's predictive ability (149, 358-361) and its responsiveness to intervention (362, 363), making it a clinically useful tool to monitor physical frailty in the context of physical activity.

In multiple regression analysis the LFI was significantly associated with both average acceleration and intensity gradient when controlled for age and refractory ascites. These findings were explored further when four groups were created based upon the four quartiles of LFI scores outlined in Chapter 2. There was no overall or between group difference in average acceleration between LFI quartiles. However, there was a downward trend from those who were most frail (4^{th} quartile, purple line) having much lower average acceleration than those who were least frail (i.e. robust) (1^{st} quartile, red line) (12.09 vs 21.97m*g*) (**Figure 5.3**). Low study numbers may be the cause for lack of significance seen here. Our results show that the most robust patients with ESLD (1^{st} LFI quartile, red line) have comparable physical activity profiles to individuals ten years their senior (mean age 56 vs 65 years) with either COPD or cardiometabolic disease (average acceleration/intensity gradient 21.9m*g*/-2.68 [ESLD 1^{st} LFI quartile] vs 29.1m*g*/-2.73 [COPD] and 22.4 m*g*/-2.73 [cardiometabolic disease]). However, those who were most physically frail (4^{th} quartile, purple line) were considerably less active (average acceleration/intensity gradient 12.09m*g*/-3.08) than those with COPD or cardiometabolic disease (313, 364). These results indicate the profound inactivity and physical frailty seen in patients with ESLD compared to other disease populations.

Despite these findings, there are no specialist physiotherapists within any of the seven UK Liver Transplant Centres funded permanently to deliver outpatient physical activity or

exercise interventions in the clinical setting (outside of research). In contrast, pulmonary rehabilitation services for patients with COPD are commissioned by Clinical Commissioning Groups (CCGs) on a local, regional and national basis, with 158 provider organisation in place across England (365). This demonstrates the distinct health inequality in terms of distribution of health resources for those with ESLD.

Additionally, unlike the strong association between intensity gradient and the LFI in our cohort of patients with ESLD, intensity of physical activity was not associated with lower cardiometabolic risk in those with chronic disease (i.e. diabetes, ischaemic heart disease). Therefore, when considering advice for lowering cardiometabolic risk in patients with chronic disease, the message "move regularly, regardless of intensity" may be given. In contrast, based on our findings to improve physical frailty, patients with ESLD may be advised "when you walk, walk briskly". These subtle, but important differences indicate the need to separate disease groups when publishing physical activity recommendations as what may be effective in one cohort, may not be in another. However, larger, longitudinal studies are needed to confirm these findings.

As one may expect, those who were the least frail (i.e. robust) participated in higher volumes of physical activity at higher intensity compared to those who were most frail. Specifically, the robust group accumulated the volume of their activity by participating in smaller bouts (≤1 minute) of higher intensity activity, indicating that short bursts of high intensity activity may be effective at reducing physical frailty in ESLD. This is supported by **Figure 5.4** which shows that for one minute of continuous activity, the robust group reached an intensity indicative of a slow run (400m*g*), whereas the most frail group only reached an intensity

indicative of a slow walk (100m*g*) for the same time period. Remarkably, the lower three quartiles (70% of the cohort) didn't reach two minutes of slow walking, highlighting again what was seen in Chapter 4, that participation in continuous activity in our cohort of patients with ESLD is far from that of the recommended 5-10 minute continuous bouts of moderate intensity physical activity advised (56). Some of the reasons for this, such as fatigue, fear of breathlessness (366), impaired mitochondrial function (288) etc., have been discussed in Chapter 4, and do require further investigation. Yet, more importantly at this stage, these findings inform us of the type of physical activity intervention potentially needed to most effectively reduce physical frailty in patients with ESLD.

High intensity interval training (HIIT) is an exercise training method which involves repeated short (<45 seconds) or long bouts (2-4 minutes) of relatively high intensity exercise alternated with recovery in the form of rest or low intensity exercise (367). Within the clinical setting it has been shown to be safe, feasible and effective in a range a patient populations (368-370). HIIT can be easily embedded into clinical pathways and uses a wide range of exercise modes, such as walking, stair climbing, cycling and resistance exercises (371). Our finding of short bursts of high intensity physical activity reduces physical frailty is supported by evidence outside of the liver field. For example, Seldeen et al. (2018) showed improvements in Fried physical frailty scores, fibre size and mitochondrial mass in 6 aged (24 month) sedentary mice who followed a HIIT programme three times a week for 16 weeks (372). Additionally, Menoto and colleagues (2007) found that high intensity walking training was significantly better than moderate intensity continuous walking in increasing thigh muscle strength, and peak aerobic capacity in middle-aged and older adults (373).

Furthermore, our results are also in accordance with findings within the liver field. For example, Debette-Gratien and colleagues found that twice weekly muscular strengthening exercise (70-80%max strength) for 12-weeks resulted in significant improvements in quadriceps muscle strength (191). Similarly, Williams and colleagues found significant improvements in muscle function (short physical performance battery) following 6-weeks of bodyweight resistance exercises (183). However, despite these improvements, one of the biggest challenges facing health care professionals is to understand how to engage patients with physical activity, with reported adherence varying from 6-100% (192, 206). Lack of engagement with physical activity may be attributed to the individual's motivation (374). It has been suggested that interventions grounded in behavioural change theory are more effective at motivating individuals to engage in physical activity than those that are not (375, 376). However, to date, there has been no incorporation of behavioural change theories into physical activity interventions for patients with ESLD, highlighting a much needed area for future research.

5.4.5 Limitations

In addition to the ones highlighted in Chapter 4 (sample size, predominantly ArLD etc.), there are other limitations to this study which restrict the applicability of the findings presented. The results provide good insight into physical activity profiles and predictors of these profiles, yet there is no comparison to clinical outcomes or the response to an intervention. Therefore, although it seems that physical activity is related to physical frailty, we cannot determine how much of an influence this has on clinical outcomes, whether it can be influenced by intervention, or indeed whether there are any changes to the patient course beyond liver transplantation.

Furthermore, physical activity is inherently going to be influenced by the patient's motivation to engage (377). The studies conducted in Chapter 4 and 5 do not investigate potential psychosocial and/or behavioural influences on physical activity participation. This would provide a more holistic understanding of physical activity in ESLD and should be considered in future studies to guide design of physical activity interventions which enable patients to engage. Additionally, the participants within this study, as well as Chapter 4, were an outpatient ambulatory cohort, which may favour those on the LT waiting list who have greater physical activity levels than those who are hospitalised during their time on the LT waiting list.

Lastly, physical activity monitoring was only taken from a single time point which may not be an accurate representation of their longitudinal engagement in physical activity. For example, data will have been hindered by those who during the two week period of wear time may have had admissions to hospital with infections, acute episode of hepatic encephalopathy or other liver and non-liver related morbidity. Considering the significant impact on physical activity outcome for those with refractory ascites, data on when they last received a paracentesis may help guide when is best to implement physical activity intervention.

5.4.6 Contributions to the field

This study is the first to analyse the associations of demographic, clinical and physical frailty factors on physical activity profiles in patients with ESLD. These results provide clinicians with valuable information on who to target when prescribing physical activity interventions, particularly within the context of the current resource limited NHS climate. Furthermore, the demonstration of physical frailty measures which are significantly associated with physical

activity (i.e. muscle strength [peak extensor quadriceps strength] and physical performance [LFI]) mean that the most useful measures can be utilised to track responsiveness to physical activity interventions.

Furthermore, our study is the first to demonstrate the impact of different physical activity profiles on physical frailty. In the limited data available thus far, it is suggested that different populations benefit from different physical activity profiling advice (257, 313, 364). Therefore, this study provides preliminary data on the type of physical activity programme that is likely to most effectively improve physical frailty in ESLD. This method will help facilitate evidence-based individually tailored physical activity programmes/recommendations specifically targeted to improve physical frailty, for example, *"when you move, move with intensity"*. This finding lends itself to programmes which focus on short bursts of HIIT activity (i.e. brisk walking) rather than longer periods of lower intensity activity such as slow walking. However, further larger longitudinal studies, specifically focused on ESLD, are needed to establish physical activity guidelines for these patients.

5.5 Conclusion

In summary, older age, presence of refractory ascites and ArLD had the strongest relationship with physical activity, indicating that these patients should have priority for physical activity interventions in resource limited clinical settings. Measurement of muscle strength and physical performance via the LFI was the most clinically useful significant predictor of physical activity and should be incorporated into physical frailty assessments, longitudinal monitoring and in the measurement of response to a physical activity intervention. The volume and intensity of physical activity is reduced relative to HC in patients with ESLD. Physical activity,

most notably the intensity of activity, is further reduced in those who are most frail. Physical activity interventions which focus on short bursts of higher intensity activity, e.g. brisk walking, as well as increasing total volume of physical activity, e.g. walking, are likely to optimise improvements in physical frailty in patients with ESLD.

CHAPTER 6:
GENERAL DISCUSSION AND CONCLUDING REMARKS

6.1 Discussion overview

There has been a vast amount of literature highlighting not only the prevalence of physical frailty but the severe detrimental effect it has on clinical outcomes, associated treatment costs and health-related quality of life in patients with end-stage liver disease (ESLD) (26, 56, 146, 149, 240, 378). However, literature originates predominantly from North America and little is known regarding the physical frailty prevalence within the United Kingdom (UK) or indeed, how to manage it. Whilst many attempts have been made to address physical frailty in ESLD through the use of physical activity interventions (206, 379), heterogeneity of physical frailty assessment tools, physical activity protocols, and a lack of robust large randomised control trials (RCT), means that the translation of evidence into clinical practice is fairly non-existent. Actually, none of the seven UK liver transplant (LT) centres currently fund physiotherapy for patients with ESLD outside of clinical research. Furthermore, there is no UK-consensus on specific tools to measure physical frailty with hand grip being the only measure of physical frailty recorded nationally within the National Health Service Blood and Transplant (NHSBT) database. Perhaps the reason for lack of investment stems from uncertainty around prevalence of physical frailty within the UK, optimal physical frailty and physical activity assessment tools, and the specific physical activity advice needed to bring about improvements in physical frailty in patients with ESLD. This thesis aimed to provide some clarity to these reservations and are discussed more widely in the sections below.

6.2 Assessment of physical frailty in end-stage liver disease (ESLD)

The term physical frailty encompasses muscle mass, muscle strength, muscle function, physical disability and aerobic capacity. Whilst low muscle mass leads to poor clinical

outcomes (56, 198, 353), the most widely used and robust measures of muscle mass, computed tomography (CT) and/or Magnetic Resonance Imaging (MRI), are costly and time-consuming. In our wider study evaluating sarcopenia in chronic inflammatory disease (ESCID), we investigated the validity of using a cheaper, more time effective and clinically useful alternative, anatomical cross-sectional area (ACSA) of quadriceps on thigh muscle ultrasound. Whilst ACSA correlated well with skeletal muscle index at the 3rd lumbar vertebrae on MRI (351), the results in Chapter 5 showed that ACSA did not predict physical activity profiles and therefore muscle mass may not be a useful measure when considering physical activity as an intervention to manage physical frailty.

The Liver Frailty Index (LFI) encompasses muscle strength, muscle function and physical performance in a battery of tests (hand grip, chair stands, balance) used to identify physical frailty specifically within ESLD (146). It is the most extensively investigated physical frailty tool to date with evidence demonstrating that it is a robust clinically useful tool in predicting outcome both pre-and post- liver transplant (LT) within North America (106, 149, 361). Furthermore, in Chapter 5, the LFI was shown to be the most robust and clinically useful measure of physical frailty to predict physical activity profiling, highlighting its potential to track change following physical activity interventions. Additionally, the results of Chapter 2 investigated the validity of the LFI in a large UK-based LT centre. The results demonstrated the profound prevalence of physical frailty in those on the LT waiting list, with less than 20% of patients presenting as robust. These results were similar to that seen in North America by Lai and colleagues and substantiates the overwhelming need for interventions to improve physical frailty in patients with ESLD (146). Like the findings thus far in North America, the results of Chapter 2 also showed that the LFI was a significant predictor of overall and LT

waiting-list mortality, highlighting the need to include the LFI in all UK LT assessment centres and ideally be collated nationally within the NHSBT database.

The one limitation of the LFI is its inability to assess aerobic capacity. Low aerobic capacity is prevalent in ESLD and is associated with mortality pre-and post-LT (64). It is an important aspect of the physical frailty assessment as it can not only inform the assessor of the ability of the patient to withstand the cardiorespiratory stress undergone during a LT, but also guide the level of prescribed aerobic-based physical activity intervention to elicit physiological change. However, formal assessment has often been bypassed due to the historical need to complete either a cardiopulmonary exercise test (CPET) or a field-walking test such as the six-minute walk test (6MWT); both of which have their clinical limitations (expense, expertise, equipment etc). The Duke Activity Status Index questionnaire, provides a quick and inexpensive alternative to CPET and field-walking tests (163). The validation of the DASI in Chapter 2 supported previous findings that low aerobic capacity is undeniably prevalent in patients with ESLD (64), but also for the first time that it significantly predicts overall and waiting list mortality in patients undergoing a LT. Consequently, the findings from Chapter 2 show that the LFI and DASI are quick, easy to use and clinically useful outcome measures that account for all aspects of physical frailty. They provide the assessor with a good overview of the patient's physical frailty status, which will help guide the need for intervention as well as the level of such intervention. Further validation of these outcomes would be achieved through the investigation of their responsiveness to physical activity interventions and their use in multiple centres European wide.

6.3 Assessment of physical activity in ESLD

Physical activity has been long known to improve multiple bodily systems and reduce risk of disease (380). Several studies have attempted to improve physical frailty in patients with ESLD (206), but heterogeneity between intervention protocols has made it difficult to translate findings into clinical practice (206). Before optimal physical activity protocols can be defined, a thorough understanding of daily physical activity profiles in patients with ESLD is needed. To date, studies investigating physical activity in ESLD have been limited to subjective questionnaires or basic outcomes such as step counts and time spent at sedentary, light and moderate to vigorous physical activity (MVPA) using accelerometer-based data (116, 273, 276). Questionnaires are self-reported instruments which quantify physical activity based on patterns of groupings of specific movements (i.e. sitting, standing, walking) (212). Whilst this provides investigators valuable information on participant behaviour, it gives very little information on the intensity that the individual is working at. In contrast, accelerometer-based monitors quantify raw acceleration signals from physical activity-associated bodily motion (224),

Nevertheless, caution should be applied when comparing studies who have used accelerometer-based data. Research to date in ESLD reports physical activity participation in categories with pre-defined cut-off points. Biaxial accelerometer data is recorded in “counts” whereby intensity and magnitude of acceleration is recorded over a set time period (211). In comparison, the GENEActiv triaxial accelerometer used within the studies in Chapter 4 and 5 records and stores acceleration data, where a standardised measure of acceleration is used (i.e. Euclidean Norm Minus One [ENMO]) (220). “Counts” are calculated using proprietary

algorithms which are developed and patented by manufacturers of the device, and data usually analysed using cut-points to create categories of time spent in different intensities of physical activity. This means that data can only be accurately compared if the same cut-off points and accelerometer devices have been used. Furthermore, limiting analysis to these specific categories means that you can only analyse small proportions of the day, for example total MVPA in our own study, and previous studies only accounts for <5% of the 24 hour period (273, 276). In contrast, describing the intensity distribution of acceleration data for the entire 24 hour period, using open-source software, generates continuous physical activity outcomes which can be compared post-hoc to different cut-points and time periods (220). Accelerometer data retrieved in this way is also comparable across any raw acceleration, including the most widely used research-based accelerometers, the GENEActiv, ActiGraph and Axivity (220), allowing for greater generalisability of findings between devices, studies and disease cohorts. Future research should aim to recruit larger sample sizes and use physical activity outcomes derived using open-source methods from raw-acceleration data to harmonise physical activity data collection to provide greater understanding of 24 hour physical activity profiles of patients with ESLD.

6.4 Prioritisation of physiotherapy led services for patients with ESLD

Prior to, and even more so since the Coronavirus-19 pandemic, the National Health Service (NHS) has been under increasing pressure to meet the growing demands (381). It is well understood that allied healthcare professionals (AHPs), including Physiotherapists, have an essential role in supporting the rest of the NHS in meeting these demands (382, 383), yet funding for such services in ESLD are still in development. In an ideal world, based on the current literature, all patients with ESLD would have access to a physical frailty assessment

and associated therapeutic interventions. However, considering the significant financial shortfall within the NHS at present (384), the need to stratify services to those most in need has never been more important. The findings in Chapter 2 and 5 provide insight into those who may benefit most from a physical frailty and physical activity assessment.

The findings from Chapter 2 showed that those who are female and/or have low sodium are at greatest risk of physical frailty as measured by both the LFI and DASI. These findings were in keeping with other studies. For example, Lai and colleagues found that in a multicentre cohort of 1405 patients with cirrhosis waiting for a LT females were more physically frail and this frailty accounted for 13% of the known waiting list mortality gender gap (385). These findings combined, emphasise the importance of clinicians highlighting to female patients their increased risk of physical frailty and identify these patients for a LFI and DASI assessment along with a potential referral to physiotherapy for physical activity intervention. Whilst there is limited literature on the impact of low sodium and physical frailty, Fujisawa and colleagues found in a cohort of 2982 elderly (≥70 years) participants that mild hyponatraemia (serum sodium 130-135mEq/L) was independently associated with gait dysfunction (Odds ratio [OR] 5.3, 95% confidence interval [CI] 1.1-25.4), p=0.04) and balance impairment (OR 2.5, 95% CI 1.2-5.5, p=0.02)(386). Whilst the mechanism for this is unclear, there are suggestions that it may be related to the loss of osmolytes, such as glutamate (neurotransmitter involved in control of movement, i.e. gait), during brain cell swelling in hyponatraemia (387, 388). In contrast, ascites was not an independent predictor of physical frailty, but it was an independent predictor of physical activity intensity distribution (intensity gradient) (Chapter 5). In a post hoc analysis it was found that those with refractory ascites (median sodium 136, IQR 134-140 mmol/L) had significantly lower sodium levels than those without/controlled ascites (median sodium 137, IQR 136-141 mmol/L; p=0.02). Moreover, only 8% of those

without or controlled ascites compared to 37% of those with refractory ascites presented with mild hyponatraemia (130-135mmol/L). While there are many other causes for physical inactivity in refractory ascites, such as the limitations associated with weight bearing with/carrying such large amounts of fluid, the associated imbalance of muscle regulation and calorie deficit, sodium levels should also be considered and managed optimally to promote improvements in physical frailty and engagement with physical activity. However, further research investigating the impact of ascites and hyponatraemia on physical frailty in ESLD are needed to fully understand the mechanisms driving these changes.

6.5 Physical activity in ESLD: what should we be advising?

The results of our study in Chapter 4 demonstrated that participants with ESLD did not participate in more than one daily minute of continuous activity at an intensity equivalent to a slow walk, indicating that any physical activity undertaken by our patients with ESLD is predominantly completed at very low intensities. Despite this, many research studies to date have encouraged patients to participate in a minimum of 10 minutes of continuous moderate intensity physical activity (183), with several encouraging 30-60 minutes (187, 192, 194, 389). Furthermore, an American expert opinion statement on exercise in sarcopenia in LT recommends that patients *"perform moderate intensity exercise for no less than 30 minutes per day"* with exercise bouts lasting *"no less than 5-10 minutes"* (56). It is therefore not surprising that there have been several reports of low adherence to physical activity/exercise interventions in patients with ESLD based on my findings (188, 189, 192, 194). Consequently, future studies should consider building up tolerance to moderate intensity physical activity starting with smaller bouts (<5 minutes) of continuous activity to facilitate long-term engagement.

If patients are only participating in very short bouts of continuous activity, attention should be given to the intensity of these minutes. For example, the results of Chapter 5 showed that intensity, rather than volume of physical activity was associated with lower physical frailty scores (LFI), indicating that it is the intensity of activity rather than the amount of activity that may improve physical frailty and consequently clinical outcomes in ESLD. An obvious consideration for future intervention design may be that of high intensity interval training (HIIT), whereby short (<45 seconds) or long (2-4 minutes) bursts of relatively high intensity exercise is alternated with recovery in the form of rest or low intensity exercise (367). Whilst HIIT has been shown to be safe and effective in other disease cohorts (368, 369, 373, 390, 391), it has not been investigated in ESLD.

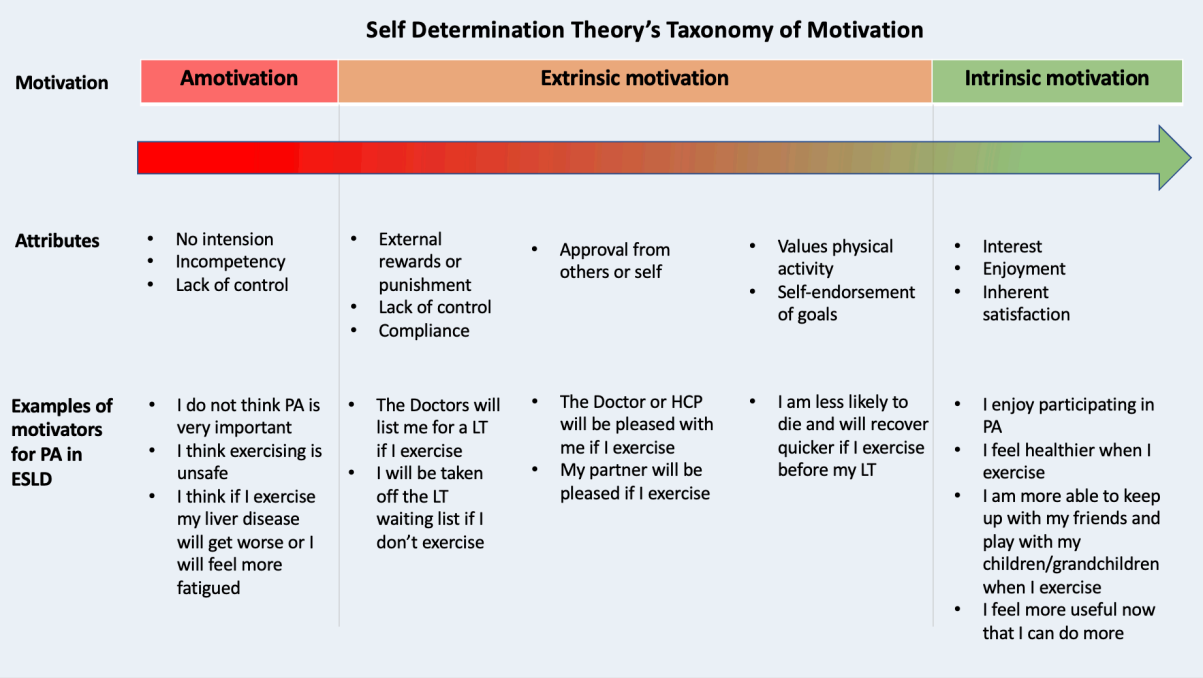
However, before designing such an intervention, one must consider what high intensity actually means for patients with ESLD. High intensity, or vigorous-intensity as it is also often referred to, is activity that requires a large amount of effort resulting in a substantially higher heart rate and respiratory rate, with the usual cut-off for vigorous intensity being 400mili-gravitational units (392). However, Kingsworth and colleagues recently suggested that using absolute intensity thresholds may not be appropriate for populations with reduced exercise capacity such as those with ESLD. In a study of 230 participants, 76 with Chronic Obstructive Pulmonary Disease (COPD), Kingsworth and colleagues asked participants to wear wrist-worn accelerometers for seven consecutive days and complete an incremental shuttle walk test (ISWT). The intensity of the most active accumulated activity for between 5 and 980 minutes (M5-M980) of the day was calculated and expressed in relative terms, as a percentage of an individual's predicted maximum acceleration during the ISWT. They demonstrated that despite the COPD group having lower intensity of activity (accelerations) across the whole

day, the activity was consistently at a higher relative intensity than the control group. By way of explanation, relative to their aerobic capacity, the COPD group completed their daily activity at a higher intensity than the controls. This suggests that current commonly used absolute intensity thresholds are unsuitable for COPD populations, and therefore are also likely to be unsuitable for patients with ESLD. Therefore, future research should aim to establish what the relative intensities are for those with ESLD and consider personalised thresholds when designing HIIT programmes. For example, whilst a HIIT programme for a healthy adult may involve short bursts (i.e. 2 mins) of running, 2 minutes of activity at an intensity equivalent to a slow walk may be just as vigorous for patients with ESLD. Personalising programmes in this way will ensure activity intensity is appropriately set for the individual as well as support ongoing adherence.

6.6 How to support adherence to physical activity in ESLD

In addition to appropriate prescription of physical activity interventions for patients with ESLD, one must consider the influence of patient motivation on long-term physical activity engagement. Psychological behavioural theories, such as self-determination theory, provide a systematic framework to identify these needs (393) and have been successfully applied to behaviour change to improve adherence to physical activity within healthcare (394-397). Self-determination theory stems from the understanding that human behaviours are influenced by personal and contextual motivational factors (398). Personal motivational factors to engage with physical activity can arise from intrinsic or extrinsic factors. Details of these factors and examples of how this might present in those listed for a LT are presented in **Figure 6.1**. Application of self-determination theory behaviour therapy aims to develop patient motivation towards physical activity from a place of intrinsic, rather than extrinsic, thought

processes to facilitate continuous engagement (377). Furthermore, the contextual motivational factors, i.e. psychological needs of the patient, need to be addressed. For example, the patient should feel a sense of choice around one's behaviour (autonomy), be able to bring about positive change in a desired outcome (competency), i.e. improvements in physical frailty, and feel accepted by one's social environment (relatedness) (377). Understanding and training in the delivery of these behaviour theories to healthcare professionals is needed to optimise delivery of physical activity-related interventions and should be considered in the design of future studies.



(Adapted from Ryan and Deci, 2017)(398)

Figure 6.1 An overview of self-determination theory including examples of how a patient with ESLD may present

6.7 Future directions

The findings of this thesis and the known limitations highlight the much needed research for the management of physical frailty in ESLD. Whilst the combination of North American data

and the data found in our study (Chapter 2) demonstrates the robustness of the LFI, validation of its use, as well as the DASI in other UK and European centres are still needed. However, the main focus for research moving forward is to develop a large randomised control trial investigating physical activity/exercise in patients with ESLD. Alongside my PhD I was a co-applicant and lead for intervention design in the successful application for a £1.5 million National Institute for Health Research (NIHR) Efficacy and Mechanism Evaluation (EME) grant (https://fundingawards.nihr.ac.uk/award/NIHR129318). This NIHR EME grant funds the randomised control trial (n=266) Home-based **EX**ercise and motiv**A**tional programme before and after **L**iver **T**ransplantation: **ExaLT trial**, which opened in May 2022. It is a dual-site study, whereby I am the Intervention/Physiotherapy Lead and University Hospitals Birmingham site Principal Investigator.

The ExaLT trial involves randomising patients to either; (1) the intervention arm, which delivers an individualised aerobic and resistance-based exercise programme (appendix 1 and 2) alongside motivational behaviour therapy, termed *"Empowering Physio"* (a combination of behavioural change techniques centred around self-determination theory) up to one year pre-LT and 24-weeks post-LT, or (2) the control arm, where participants receive a one off advice leaflet both pre-and post-LT. Participants are followed up on a 6-weekly basis until time of LT and again, 6-weekly post-LT until 24-weeks post-LT. Following extensive input from Patient and Public Involvement and Engagement (PPIE), the physical component score (PCS) of the Short-Form-36 (SF-36) at six-months post-LT was chosen as the primary outcome. Patients unanimously felt that it was not enough to survive the LT but to survive well, placing a health-related quality of life outcome measure at the centre of our findings. Secondary outcomes include the LFI, DASI, accelerometery, behaviour psychology questionnaires and

anthropometry are being collected. Furthermore, a sub study of n=100 participants will also partake in a CPET, 6MWT, thigh muscle ultrasound and provide serum muscle biomarkers at baseline, 6-weeks (pre-LT), and 24-weeks post-LT. An overview of the ExaLT study design can be found in **Figure 6.2** and the full protocol is provided in appendix 3.

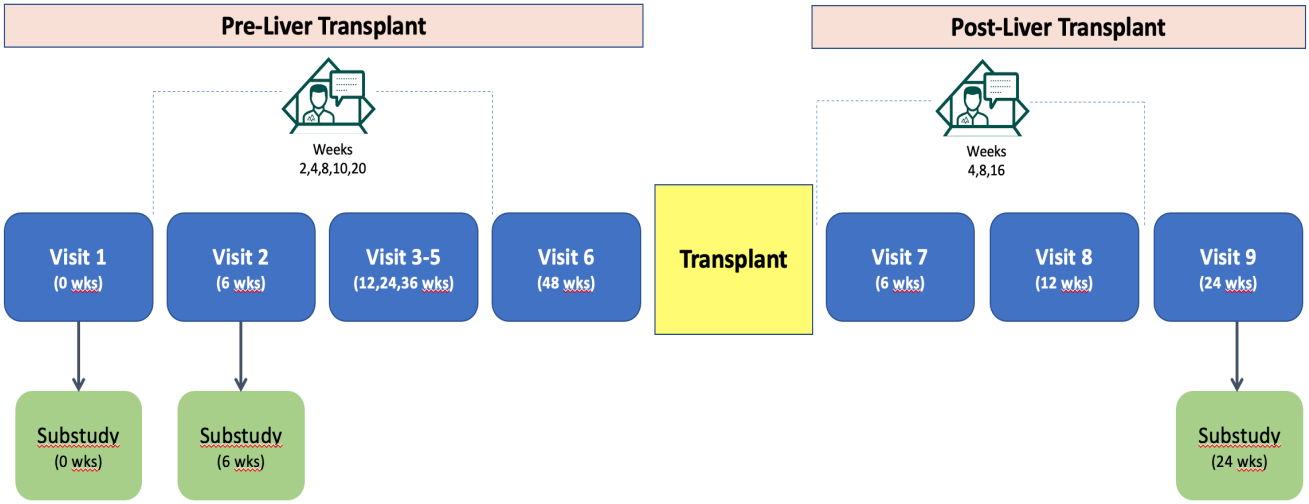


Figure 6.2 An overview of the ExaLT trial study design
Assessments completed at visits 1-9: SF-36, LFI, DASI, anthropometry, Health Care Climate Questionnaire (perception of need support of physiotherapist), Basic Psychological Need Satisfaction in Exercise Scale (assesses psychological needs (i.e. feelings of autonomy, competence, relatedness) of participant), and Behaviour Regulation in Exercise Questionnaire-2 (assesses the participant's degree of self-determined motivation to engage in exercise)
Assessments completed as part of the sub study (n=100): CPET, 6MWT, thigh muscle ultrasound, serum muscle biomarkers**Notes:**
Telehealth calls completed at weeks 2, 4, 8, 10, 20 (pre-LT) and weeks 4, 8, 16 (post-LT) for intervention arm only
Participants can be transplanted at any time point after visit 1 and will move to the post-LT visit pathway (i.e. visits 7-9) accordingly

In relation to the themes discussed in this thesis, the ExaLT trial will contribute substantially to future literature for the management of patients with ESLD undergoing a LT. These contributions will be made in the following ways:

1. the assessment of physical frailty by:
    a. investigating the ability of the LFI and DASI to respond to a physical activity/exercise intervention
    b. investigate the ability of the LFI and DASI to predict post-LT outcomes in a UK cohort
    c. correlate the DASI with gold standard objective measures of aerobic capacity (i.e. VO_2peak and anaerobic threshold on CPET)
2. the assessment of physical activity in patients by:
    a. investigating the ability of physical activity profiles to predict clinical outcomes (i.e. mortality, hospitalisation, infections, readmissions)
    b. investigate relative intensities of physical activity compared to maximal acceleration achieved in CPET and/or acceleration during 6MWT
3. the impact of physical activity/exercise on:
    a. measures of physical frailty including PCS of SF-36, LFI, DASI, 6MWT and CPET
    b. mental health as measured by the mental component score (MCS) of the SF-36
4. the impact of psychological components on adherence to physical activity/exercise interventions:
    a. how the type of participant motivation influences adherence to physical activity/exercise
    b. how the use of *"Empowering Physio"* can influence behaviour change

6.8 Summary

In summary, physical frailty is highly prevalent in a UK-based cohort of patients with ESLD. The results of this thesis demonstrate the clear need for accurate assessment of physical frailty and investment in services to manage it. The LFI and DASI are quick, easy to use, robust measures which can identify those who are physically frail, as well as predict clinical outcome. Physical activity-based interventions are needed to improve physical frailty in those with ESLD. Prior to developing these interventions, a thorough understanding of 24 hour physical activity profiles in patients with ESLD is needed. Use of triaxial, evidence-based accelerometers such as the GENEActiv, facilitate in depth analysis of these profiles and should be incorporated into future physical activity-based trials. From the results presented in this thesis, it is suggested that physical activity interventions involving short bursts of high intensity (relative to capacity) physical activity is likely to yield greatest improvements in physical frailty measures, yet further research with a larger sample size is needed. The results of the ExaLT trial will contribute significantly to the gaps highlighted in this thesis thus far. Analysis of this large randomised physical activity-based trial will provide greater understanding of the utilisation of physical activity to manage physical frailty and will help guide future clinical services.

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APPENDICES

Appendix 1 Aerobic exercise component of the ExaLT home-based exercise programme (HBEP)

The initial level (duration, recovery period, intensity) of aerobic exercise sessions will be determined from the baseline DASI (**Figure 8.1**), while accounting for any exercise limiting comorbidities, such as ascites, peripheral oedema and/or hepatic encephalopathy. It will be recommended to the participants that they aim to complete two sessions of aerobic exercise per week. In line with *Empowering Physio* theory, a rationale for this recommendation will be provided. The participants will also be asked to select their exercise modality of choice from the following options; walking, cycling, swimming, cross-trainer, rowing ergo or running. In collaboration with the physiotherapist, the participant will be able to change their choice of modality week by week or continue with the same choice depending on their preferences. Furthermore, the physiotherapist will involve the participant in discussions about previous positive exercise experiences to facilitate personal goal setting. The level set will be appropriate to the participant's current level of function but also ensure the participant feels competent in their exercise effort. Each aerobic session will consist of alternating "work" and "active rest" periods:

- During the work periods, participants will be asked to exercise at a moderate intensity (rate of perceived exertion (RPE) score of 12-14 (6-20 scale).
- During their active rest periods, participants will be asked to work to a RPE of 9-11. Details of the aerobic exercise intervention and levels of difficulty are detailed in **Table 8.1**.

Exercise intensity will be progressed depending on the feedback from regular Telecalls to the participant (weeks 2, 4, 8, 10, 16 and 20).

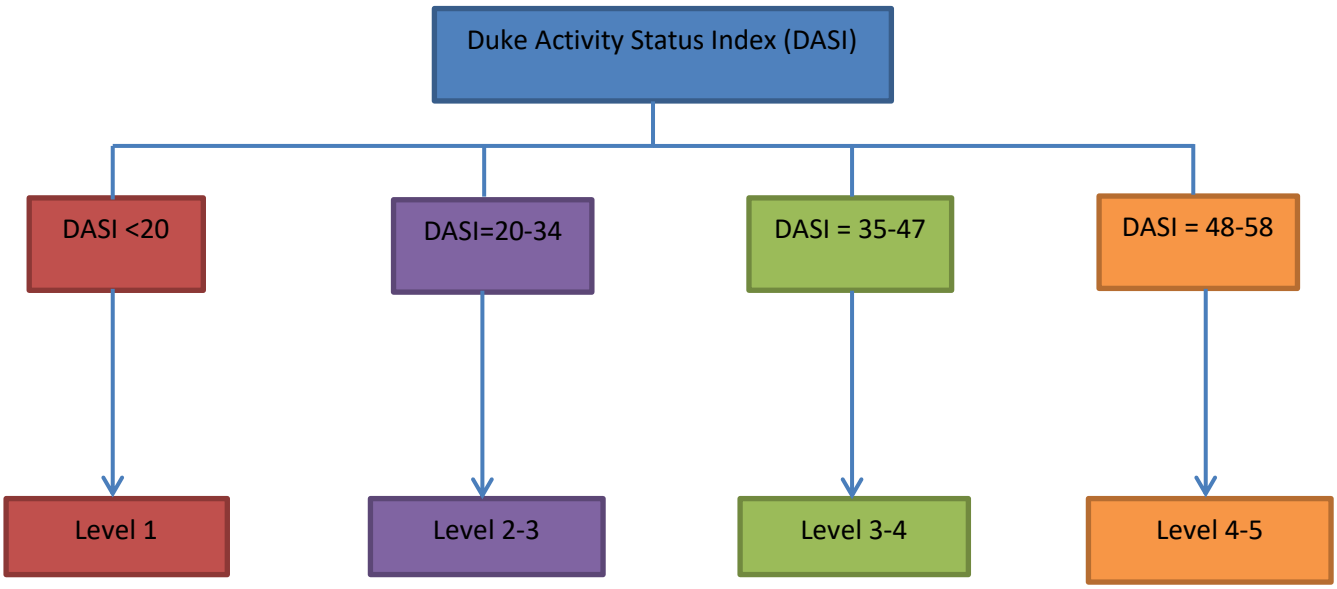

Figure 8.1 - Flow diagram for use of DASI when prescribing the 'entry level' aerobic exercise programme

Table 8.1: ExaLT aerobic exercise programme

Level of difficulty	Exercise	Intensity	Duration (mins)
1	Walking/cycling/swimming/cross-trainer/running	1x2mins @ RPE 12-14 1x3mins recovery @ RPE 9-11 1x2mins @ RPE 12-14	7
2	Walking/cycling/swimming/cross-trainer/running	1x3mins @ RPE 12-14 1x3mins recovery @ RPE 9-11 1x3mins @ RPE 12-14	9
3	Walking/cycling/swimming/cross-trainer/running	1x5mins @ RPE 12-14 1x3mins recovery @ RPE 9-11 1x5mins @ RPE 12-14	13
4	Walking/cycling/swimming/cross-trainer/running	1x7mins @ RPE 12-14 1x3mins recovery @ RPE 9-11 1x7mins @ RPE 12-14	17
5	Walking/cycling/swimming/cross-trainer/running	1x10mins @ RPE 12-14 1x3mins recovery @ RPE 9-11 1x10mins @ RPE 12-14	23
6	Walking/cycling/swimming/cross-trainer/running	1x15mins @ RPE 12-14 1x3mins recovery @ RPE 9-11 1x10mins @ RPE 12-14	28
7	Walking/cycling/swimming/cross-trainer/running	1x20mins @ RPE 12-14 1x3mins recovery @ RPE 9-11 1x15mins @ RPE 12-14	38
8	Walking/cycling/swimming/cross-trainer/running	1x20mins @ RPE 12-14 1x3mins recovery @ RPE 9-11 1x20mins @ RPE 12-14	43
9	Walking/cycling/swimming/cross-trainer/running	1x35mins @ RPE 12-14	35
10	Walking/cycling/swimming/cross-trainer/running	1x40mins @ RPE 12-14	40

Appendix 2 Resistance exercise component of the HBEP

Participants will be asked to participate in a 20 minute circuit of bodyweight resistance exercises twice weekly on alternate days to the aerobic sessions. The circuit will consist of four cycles of 8-12 repetitions of five exercises, chosen by the patient (**Table 6.2** and **6.3**) with two minutes of "active rest" (walking slowly on the spot) between each exercise and each cycle. The programme and entry level will be developed according to baseline LFI (**Figure 6.4**), and a trial of 8-12 repetitions exercises within the designated entry level. Furthermore, the entry level will be discussed collaboratively with the participant to support feelings of competence and autonomy.

The participant will be instructed to terminate each set of an exercise when they reach a "repetitions in reserve" (RIR) of 1-2; that is, they feel they could complete 1 or 2 additional repetitions, but no more. The participant will be advised to progress to each level of difficulty once they can achieve 12 repetitions with 1-2 RIR and depending on feedback from the Telecalls at weeks 2, 4, 8, 10, 16 and 20. Details of the resistance exercise circuits and levels of difficulty are detailed in **Table 6.2** and **6.3**

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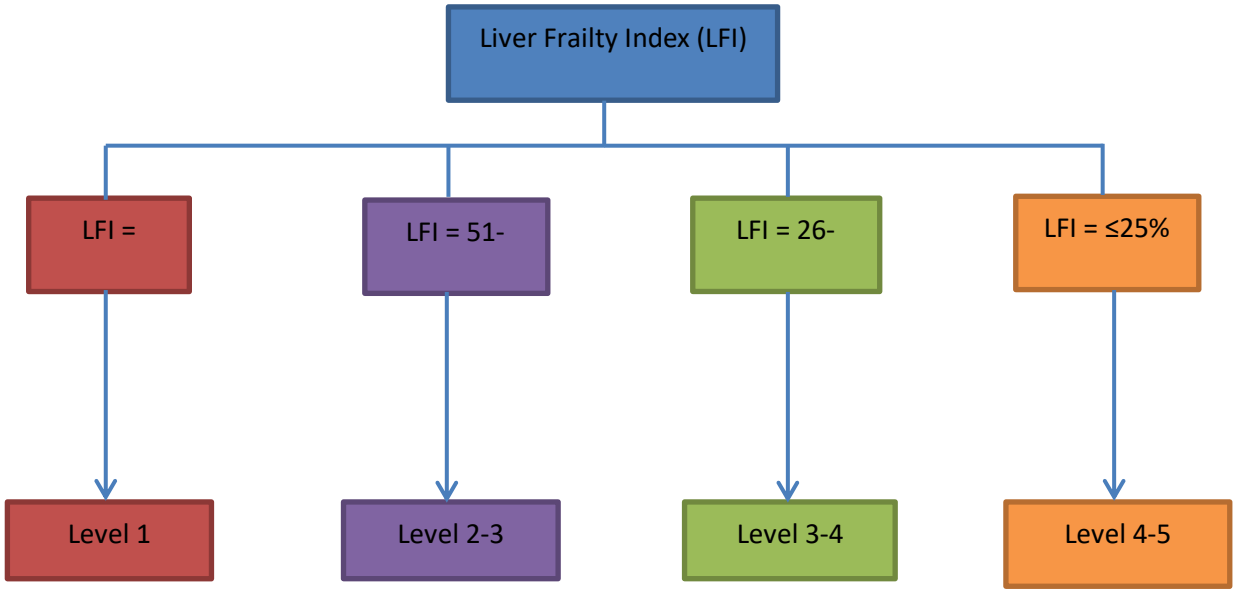


Figure 8.2 - Flow diagram for use of LFI when prescribing the 'entry level' resistance exercise programme

Muscle Group	Exercise	1	2	3	4	5	6
Upper Limb Press	**Horizontal press**	Wall press-up	Press-up on knees	Hands-elevated press-up	Progressively lower hands-elevated press-up	Press-up	Press-up with progressive band resistance
	Vertical press	Overhead press, arms only	Overhead press with light weight (e.g. soup cans)	Overhead press with heavier weight (e.g. water bottles)	Pike push-up, hands on raised surface	Pike push up	Pike push-up, feet elevated to knee height
Upper Limb Pull	**Horizontal pull**	Two-arm row with light weight (e.g. soup can)	One-arm row with light weight (e.g. soup cans)	Two-arm row with heavier weight (e.g. water bottles)	One-arm row with heavier weight (e.g. water bottles)	Two-arm row with progressive band resistance	One-arm row with progressive band resistance
	Lateral/Vertical pull	Lateral rotation with yellow TB	Bilateral abduction with TB	Diagonal TB pull	Vertical pull down with yellow TB	Vertical pull down with red TB	Vertical pull down with green TB
Lower Limb	**Squat**	Raised surface chair stands	Wall squat	Chair stands	Full squat	Squat with light weight (e.g. soup cans)	Squat with progressive band resistance
	Lunge	Static lunge with support	Static lunge without support	Dynamic half lunge	Dynamic full lunge	Walking lunge	Walking lunge with progressive load
	Step ups	Low step-up (e.g. 1 stair)	Low step-up with knee raise	Low step-up with knee raise and light weight (e.g. soup cans)	High step-up with high knee	High step-up with high knee and light weight (e.g. soup cans)	High weighted step up with high knee and progressive load
Core Stability	**Anti-anterior flexion**	Four-point kneeling holds	Four-point kneeling with leg raises	Four point kneeling alternate arm and leg raises	Kneeling plank	Plank	Plank with progressive load
	Glute med/anti-lateral flexion	Clams	Clams heels raised	Straight leg clam	Elbows-elevated side plank	Elevated side plank	Side plank
	Extension	Pelvic tilt in crook lying	Bridges	Bridges with yellow TB	Bridges with red TB	Bridges with red TB and heel raise	Bridges with red TB straight leg reps

Table 8.3 – ExaLT Resistance Exercise Session

Nº of exercises	Nº of circuits	Repetitions	Rest period between circuits (mins)	Total time (mins)
1x upper limb push **1x upper limb pull** **2x lower limb** **1x core/balance**	4	8-12	2	26

TRIAL PROTOCOL

HOME-BASED EXERCISE AND MOTIVATIONAL PROGRAMME BEFORE AND AFTER

LIVER TRANSPLANTATION: ExaLT Trial

A PHASE IIb, RANDOMISED-CONTROLLED, TWO-CENTRE CLINICAL TRIAL ON THE EFFICACY OF A HOME-BASED EXERCISE AND MOTIVATIONAL PROGRAMME IN PATIENTS BEFORE AND AFTER LIVER TRANSPLANTATION

This protocol has regard for the HRA guidance and is compliant with the SPIRIT guidelines (2013)

TRIAL SUMMARY

Title

ExaLT: Home-based EXercise and motivAtional programme before and after Liver Transplantation.

Objectives

The primary aim is to investigate whether a remotely monitored 'home-based exercise and theory-based motivation support programme' delivered by physiotherapists before and after liver transplantation (LT) (intervention group) improves quality of life (QoL; physical component score of SF-36v2) in LT recipients compared to a control group using a patient 'exercise' advice leaflet (control group).

The secondary aims are to investigate whether a remotely monitored 'home-based exercise and theory-based motivation support programme' delivered before and after LT (experimental arm) improves:

- Surgical complication after LT (comprehensive complication index (CCI))
- Mental wellbeing/health (mental component score (MCS) of SF-36v2 health-related QoL)
- Clinical markers of physical frailty and fitness (liver frailty index [LFI] ; Duke activity status index [DASI])
- Pre-LT: morbidity (United Kingdom model for end-stage liver disease (UKELD), model for end-stage liver disease – sodium (MELD-Na), hospital admissions) and mortality
- Post-LT: length of intensive care unit (ICU)/hospital stay, hospital re-admissions and mortality (30, 90, 180 day, 1 year)
- Habitual physical activity levels (daily time spent in light, moderate and vigorous intensity physical activity)
- The frequency, intensity and duration of exercise ('dose') completed
- Adherence to home-based exercise programme (HBEP) (*intervention arm only*)
- Perceptions of the health care climate (how need supportive/empowering the physio is)
- Basic psychological need satisfaction (i.e. feelings of autonomy, relatedness, competence)
- Self-determined motivation to exercise

The mechanistic objectives are to investigate:

1. What is the dose-dependent effect of the HBEP on physical fitness, muscle biology (including oxidative stress and inflammation) and their association with QoL?
2. How does the theory-based motivation support affect adherence and engagement with the HBEP?

Trial design

A phase 2b, open-label, two-centre randomised controlled clinical trial (RCT), with 1:1 individual participant randomisation.

Participant population and sample size

Adult patients (aged 18 years and over) who are awaiting a cadaveric, primary LT. Sample size = 266 patients (133 patients in each arm).

Setting

The ExaLT Trial will be based at the LT units of the Queen Elizabeth University Hospital, Birmingham (QEUHB) and the Royal Free Hospital, London (RFH).

Eligibility criteria

Inclusion criteria

- Adult patients (aged 18 years and over)
- Awaiting a cadaveric, primary LT at two LT centres: QEUHB and RFH.
- Being an out-patient at the time of baseline trial visit (consent)

Exclusion criteria

- Patients awaiting super-urgent LT, multi-organ transplantation, live-related donor LT, regraft LT
- Inability to safely comply with the exercise intervention due to:
  - severehepaticencephalopathy
  - oxygen-dependenthepato-pulmonarysyndrome
- Patients without liver failure including:
  - livercancerintheabsenceofcirrhosis
  - polycysticliverdisease
  - raremetabolic/geneticconditions.
- Patient refuses or lacks capacity to give informed consent to participate in the trial, at the point of study visit 1 (baseline)[1]

Interventions

Eligible participants will be randomised 1:1 to receive either:

Group 1: Intervention group. Remotely-monitored home-based exercise and theory-based motivation support programme whilst on the LT waiting list (max. 12 months) through to 24 weeks post-LT.

Group 2: Control group. Patient exercise advice leaflet before and after LT.

The interventions will be delivered to the participants in two phases: phase 1 pre-LT (maximum 52weeks) and phase 2 post-LT (24 weeks). The study intervention will be of variable duration pre-LT, due to the unpredictable nature of the timing of LT (median waiting time 72 days (95% CI 64-80) registered between 2018-2021). All patients that are transplanted within 52 weeks of randomisation will receive a fixed 24 week intervention after LT. Group 1 and 2 will contain approximately the same proportion of age groups, disease severity (UKELD), gender, trial site and participation rates in the 'muscle sub-study' as a result of minimisation.

[1] During the course of the trial, some participants may lose capacity because of complications of their liver condition(s), for example hepatic encephalopathy.

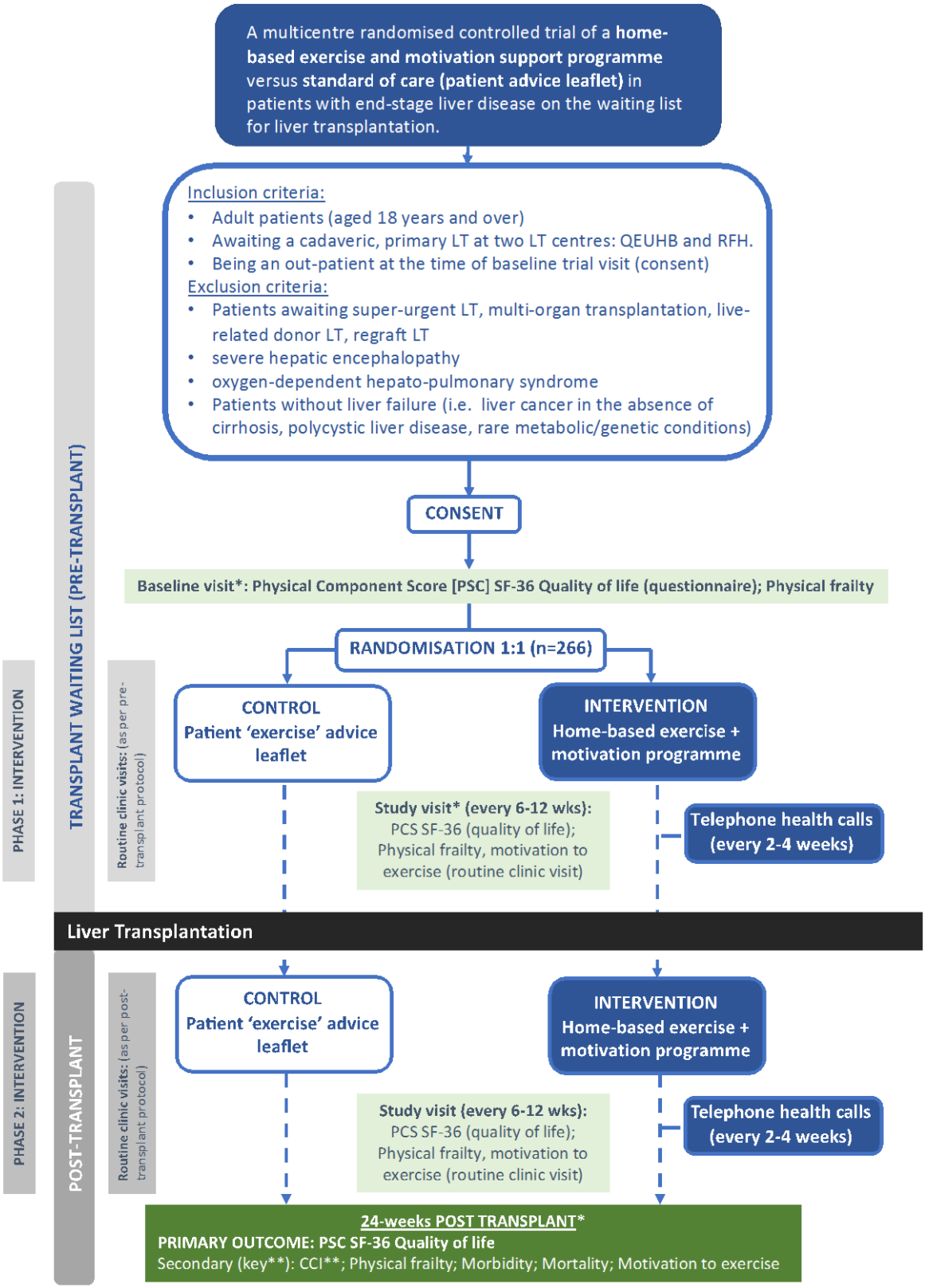
Outcome measures

The primary outcome measure is the physical component score (PCS) from the short form-36 version 2.0 (SF-36v2) health-related QoL questionnaire at 24 weeks post LT.

The 'key' secondary outcome measure is the CCI at 24 weeks post LT.
The other secondary outcome measures to be assessed at 24 weeks post LT (*_unless stated_*) include:

- MCS score of SF-36v2 health-related QoL questionnaire
- Liver Frailty Index (LFI), Duke Activity Score Index (DASI)
- Pre-LT morbidity (UKELD, MELD-Na, hospital admissions) and mortality (*_assessed up to day of LT_*)
- Post-LT length of ICU/hospital stay and hospital re-admissions (frequency, duration [days])
- Post-LT 30, 90, 180 and 365 day mortality
- Habitual physical activity levels (daily time spent in light, moderate and vigorous intensity physical activity)
- "Dose" of exercise completed (measure of the frequency, intensity and duration of exercise)
- Adherence to HBEP (*_intervention arm only_*)
- Perceptions of the health care climate (how need supportive/empowering the physiotherapist is)
- Basic psychological need satisfaction (i.e. feelings of autonomy, relatedness, competence)
- Self-determined motivation to exercise

ExaLT trial: study design



* At baseline, at 6-weeks (pre-transplant) and 24-weeks after transplant (mechanistic 'muscle' sub-study; n=100; optional): Cardiopulmonary exercise tests, muscle ultrasound and biomarkers. Key: CCI, Comprehensive complications index; PCS, Physical Component Score

1. BACKGROUND AND RATIONALE

1.1. Background

Liver disease, the third commonest cause of death in the UK, predominantly kills between the ages of 18 and 65. This leads to the loss of 62,000 years of working life each year in the UK. Liver transplantation (LT) remains the only curative treatment for patients with liver failure and the number of transplantations in the UK has risen over the past seven years by 50% to 1014/year.(1) LT is a highly resource intensive procedure requiring a large investment of healthcare resources. The average cost per procedure is estimated at over £1.1m, which includes pre-LT work-up, surgery, perioperative care, and an estimated seven year postoperative follow-up.(2) Complications whilst on the waiting list and in the perioperative period contribute substantially to this cost, and their likelihood is increased markedly by the presence of physical frailty.(3-5)

Despite a new organ allocation system and advances in clinical management, 5-7% of patients on the waiting list die before LT, largely as a result of disease severity and physical frailty.(6) LT exerts a phenomenal physiological and psychological stress on recipients who are frail as a result of long-standing liver failure. As a consequence, a further 5% of patients die within 6 months after LT.(6) Among those who survive, readmission rates are around 50% and perioperative complications can lead to prolonged hospital stays and long-term disability.(4, 5, 7) Ultimately, this results in a reduced long-term quality of life (QoL) and delayed/reduced return to productive employment after LT.(8, 9) End-stage liver disease triggers complex pathological changes in skeletal muscle, leading to sarcopenia characterised by low muscle mass and function.(10) Along with poor nutrition and physical inactivity and their close causative relationships, sarcopenia contributes to a high prevalence (70%) of physical frailty.(11) In turn, frailty is associated with poor clinical outcomes, including increased hospitalisation and intensive care unit (ICU) utilisation, (12-14) a 50% risk of severe postoperative complications(15) and a two-fold increase in pre- and post-LT mortality.(3, 16, 17) Frailty both before and after LT is associated with poor psychological and physical health-related QoL,(18-20) which is itself an independent predictor of mortality.(21) QoL post-LT significantly lags behind that of the general population (22) and although the majority are under 65 years old, fewer than 50% return to employment, which is largely attributed to prolonged disability/frailty.(9)

1.2. Trial rationale

Exercise interventions have been shown to be effective in other fields of medicine including prior to elective major surgery. However, due to the life-threatening, multi-systemic effects of end-stage liver disease, patients awaiting LT are often perceived as ‘too sick’ to exercise by healthcare professionals and the patient/carers themselves (PPI/Expert observations); with virtually no published data to support the benefits and safety of exercise in this cohort. Effective exercise interventions that reduce frailty pre- and post-LT have the potential to improve clinical outcomes and long-term QoL for this patient group, leading to cost savings for the NHS. Furthermore, a better understanding of how exercise works (i.e. on the muscular and cardiopulmonary systems) and how it can be effectively delivered (i.e. motivational approach adopted) in this unique cohort, will guide future exercise prescriptions (‘type’, ‘dose’, ‘duration’, ‘motivational strategies’) that are required to maximise the efficiency and longevity of this life-changing surgery. In an environment of substantial NHS resource limitation, identifying simple, cost-effective and remotely monitored home-based interventions should be a priority in those patients who may benefit the most.

1.2.1. Justification for participant population

For those patients awaiting LT, the benefits of exercise are unknown as traditionally they have been viewed as 'too sick' to exercise. Healthcare professionals and research teams have therefore been reluctant to use exercise as a 'medicine' in this group. What underlies this myth is that due to their underlying liver disease these patients are frequently deconditioned with substantial functional impairment,(23) which tends to be proportional to the severity of disease.(24) End-stage liver disease is a multi-system disorder leading to physical frailty (muscle wasting, weakness, poor functional status, dependence of activities of daily living), cirrhotic cardiomyopathy, malnutrition, ascites, encephalopathy, anaemia and impaired pulmonary gas exchange, all of which limit a patient's ability to exercise. Indeed, patients awaiting LT are some of the sickest and frailest patients in the NHS, to the extent that a 57 year old end-stage liver patient has the predicted physical frailty of >80 year old in the community.(25) Furthermore, there is an innate fear and anxiety (which has been confirmed by our patient feedback workshops) that exercise may actually exacerbate the complications of cirrhosis, thereby worsening a patient's quality of life (QoL) and potentially even preventing them from being eligible for LT. These factors make patients with end-stage liver disease awaiting LT a unique cohort of patients in whom virtually no data exist to support the benefits and safety of exercise (p)rehabilitation. It cannot be assumed that because a preoperative exercise programme improves aerobic capacity in relatively well patients awaiting colorectal/cancer surgery that the same is true for patients with a life-threatening multi-system disease such as cirrhosis. When a patient with end-stage liver disease requires any surgery other than LT, it is highly likely that they would present 'too' high a risk (of postoperative morbidity and mortality) to be operated on. Thus, there is pressing need for detailed studies to answer efficacy and mechanistic questions unique to this patient population that cannot be addressed by simply transferring findings from other conditions.

In order to optimise outcomes from LT these frail patients must survive their illness for an undefined period on the waiting list and then be in the best condition to survive one of the most physiologically and mentally challenging operations in the NHS. There is a theoretical case for the use of exercise therapy to improve outcomes in this cohort but this needs to be tested by rigorous clinical studies that are currently lacking.

The physical hurdles to exercise in liver failure are apparent, however, the psycho-behavioural hurdles are also poorly understood. Little is known about the motivation to engage and adhere to exercise in all chronic medical conditions and such knowledge is crucial in achieving benefits of exercise. Common psychological barriers to exercise in patients with chronic disease, include low self-efficacy (competence) and a lack of individualised support. Both of these factors contribute to low motivation to engage and adhere to exercise, and are amplified in patients awaiting LT due to patient and healthcare professional fear of causing harm. To promote optimal behaviour changes towards exercise adoption, NICE recommends that interventions target recognised determinants of behaviour (such as motivation) and are theoretically grounded.(26) An example of such a theoretical approach is self- determination theory (SDT), which centres on the determinants and positive consequences linked to autonomous motivation for exercise. SDT has been successfully applied by our research group in patients with chronic arthritis.(27) To date, the efficacy and mode of action of theory-based behaviour change/motivational interventions have not been tested in patients with end-stage liver disease awaiting and/or recovering from LT. Furthermore, training selective members of the pre-existing NHS workforce (i.e. surgery physiotherapists) to deliver the exercise intervention in a more motivationally adaptive manner can be an evolving cost-effective approach and unique to patients awaiting major surgery and/or with severe liver disease.

1.2.2. Justification for design

The ExaLT study is a phase 2b open-label two-centre randomised controlled trial (RCT) of 266 patients with end-stage liver disease evaluating the effectiveness of a unique remotely monitored pre- and post-LT programme of home-based exercise and theory-based motivation support in improving QoL post-LT. Delivering an effective home-based exercise programme that can be monitored and objectively evaluated is essential to patients with end-stage liver disease. Ensuring optimal uptake and adherence to such programmes is critical to realise meaningful improvements in health and wellbeing. The ExaLT trial intervention is designed to promote higher quality of motivation for exercise (i.e., more autonomously motivated), leading to sustained engagement with the home-based exercise program and exercise in general.

To the best of our knowledge there are no other RCTs investigating the combined effect of exercise and targeted behavioural change/motivational strategies before or after LT. Currently an American team are recruiting 500 patients, either pre-LT or post-LT at baseline, to a trial of low-level resistance exercise via a DVD versus standard advice from their physician in clinic, on physical frailty using the liver frailty index (clinicaltrials.gov NCT02367092). The ExaLT study, however, is unique in that it follows the patient through the whole LT journey (pre-LT to post-LT) and assesses key patient-reported outcomes (i.e. physical and mental components of quality of life). In contrast to the American study, it incorporates an individualised resistance and aerobic exercise programme, physiotherapy-led training/monitoring, analysis of the effects of exercise on muscle physiology, and detailed assessment of the theory-based motivation programme and its impact on adherence and engagement with exercise. In addition, there are no directly competing trials regarding lifestyle/exercise/behaviour interventions in patients pre- and post-LT in the recruiting LT units. There are a growing number of interventional trials in LT, most notably donor organ optimisation with machine perfusion techniques (i.e. the Hope trial, NAPLES study). However, we do not feel that co-enrolment will influence the results of the ExaLT trial and most importantly, the use of machine perfusion will be captured in the trial database, as will other donor factors (age, type of organ, cold ischaemic time, intra-operative complications etc).

Currently in the UK, there is a lack of standardisation for exercise/physical advice across the 6 LT units; confirmed by a national LT audit we carried out in 2018 on behalf of British Liver Transplant Group (BLTG). The national audit highlighted that clinical guidance regarding physical activity can vary between exercise advice leaflets, verbal encouragement to keep active from clinicians and, at most units, no advice at all. Whilst provision of an exercise advice leaflet is not standard of care for all clinicians, advice about exercise is recognised as “best practice” by the NHS. Consequently, increased emphasis is now being placed on the importance of communicating the benefits of exercise to patients (e.g., “Moving Medicine” – Public Health England and Faculty of Sport and Exercise Medicine). In order to standardise care for the control group across the two ExaLT trial sites (Queen Elizabeth University Hospitals Birmingham [QEUHB] and Royal Free Hospital London [RFH]) and minimise any potential variation in advice, a specifically formed ‘generic’ patient information exercise leaflet will be utilised for the control arm at both the QEUHB and RFH transplant centres.

Eligible participants will be individually randomised in a 1:1 ratio to receive either remotely-monitored home-based exercise and theory-based motivation support programme (intervention arm) OR a standardised patient exercise advice leaflet (control arm) whilst on the LT waiting list (max. 52 weeks) through to 24 weeks post-LT. Randomisation will be performed using minimisation method with minimisation variables age (≤55 years, >55 years), gender (male, female) and disease severity (UKELD ≤54, >54), trial site (QEUHB, RFH), as they are potential confounding factors. We will

also include 'consent for the muscle sub-study' (Yes, No) as a minimisation variable in order to ensure equal representation of participants in the intervention arm (Group 1) and control arm(Group 2) .

The efficacy of the home-based exercise and theory-based motivation support programme on QoL (primary end-point of the trial) will be assessed at 24 weeks post-LT. At this time-point, investigators will also be able to assess and report the safety and effects of the exercise/motivation intervention on clinical measures, including physical frailty/fitness and post-LT surgical complications, length of ICU/hospital stay and 30, 90 and 180 day mortality.

1.2.3. Justification for choice of intervention(s)

In 2014 the American Society for Transplantation set out a research agenda for exercise interventions in patients awaiting solid-organ transplantation.(28) Despite the higher numbers of LT, as compared to heart and lung, the application of exercise training in this population is virtually non-existent.

We carried out a literature review (29) to summarise the impact of physical exercise in patients with chronic liver disease through to LT. The majority of studies were small (1-50 patients), focused on supervised, hospital-based aerobic exercise interventions (but not resistance exercises) and largely excluded patients with significant liver failure needing LT.(30-32) Our work in this field has demonstrated that a supervised regimen of outpatient hospital-based exercise training sessions over 6 weeks is both feasible and beneficial to patients awaiting LT (n=9).(33) However, this model is neither scalable nor cost effective because each LT unit cares for patients over a large geographic area and for many patients the time and cost required to travel to their LT centre several times each week is prohibitive.(33-35)

Seven non-UK studies (4 RCTs; 3 observational studies) have demonstrated that supervised aerobic exercise after LT improves aerobic capacity, muscle mass/strength and in two studies, trends towards improved QoL.(36, 37) These small, heterogenous studies suggest that combined aerobic and resistance-exercises yield the most promising improvements, but adherence is challenging. We carried out a proof-of-concept pilot study of a novel home-based exercise programme in patients awaiting LT.(38, 39) 18 patients underwent 12 weeks of resistance and aerobic exercises, with weekly telephone health calls. The intervention was safe and showed trends towards improved physical frailty and QoL in patients on the LT waiting list.

There are clear advantages to home-based exercise programmes (40, 41), including increased flexibility and reduced travel burdens for patients, but it is essential that we focus on patients' motivation to engage and psycho-behavioural barriers to exercise in order to optimise such interventions delivered at home. The need for behaviour change/motivational interventions has been repeatedly emphasised by our LT patient and public involvement (PPI) groups. Despite this, no studies to date have combined exercise with motivational interventions to increase intervention adherence. Understanding the social psychological processes through which motivational intervention influences patients to adopt and sustain positive changes in home-based exercise behaviour will guide larger studies of efficacy and cost effectiveness in this field. This is a key component to ensuring the long- term success of home-based tailored-made exercise interventions outside of the secure, supervised hospital environment.

1.2.4. Justification of choice of primary outcome

The SF-36v2 (which incorporates the physical component score [PCS]), is a validated, robust, reproducible patient-reported outcomes tool for assessing QoL before and after medical/surgical interventions. It is the most widely cited QoL assessment tool in the published literature for solid-organ transplantation and chronic liver disease.(9, 21, 36, 37, 42-44) The liver and transplant PPI groups (including disease support groups, National Health Service Blood and Transplant [NHSBT]) and the patient co-applicants strongly felt that the SF36v2 PCS QoL score captures the whole transplant experience from being on the transplant waiting list through to the LT and the recovery 24 weeks afterwards. The SF-36v2 PCS has been shown to strongly correlate with physical frailty, poor functional status and complications in patients undergoing LT.(9, 21) Fundamentally to the patients, their families and caregivers, QoL is the most important outcome to them in life (i.e. in their words 'there is no point prolonging life with transplantation, if your quality of life is not worth living for afterwards'). The vast majority of patients undergoing LT are of working employment age with young families. If, however, they fail to recover their functional independence and physical activity levels post-transplant (only 2 out of 5 are deemed robust 1-year post liver transplant (45)), it has deleterious effects on their self-motivation, mental/physical health, ability to work, finances and family commitments.

The SF-36 questionnaire includes 36 questions composed of 8 multi-item scales, which reflect the impact of health problems on both the physical and mental condition of the patient. A greater score reflects better QoL. Two summary sub-scores can be calculated which are weighted combinations of the eight scales, one to reflect the impact on physical function (PCS) and one to reflect the impact on psychological function mental component score (MCS).(21) A low PCS, rather than MCS, of SF-36v2 has been associated with low survival, employment and functional status in our patient population.(9, 21) Overall, we felt that the PCS was the best outcome measure in the evaluation of experimental interventions targeting physical frailty, functional status and health wellbeing in our patient population. We also deemed an RCT powered to detect survival differences at 6-12 months post-LT as the primary end-point would not have been feasible (based on huge sample size, cost), as survival rates are consistently >90%.

1. AIMS AND OBJECTIVES

1.1. Primary Aim:

The primary aim of the study is to investigate whether a remotely monitored 'home-based exercise and theory-based motivation support programme' delivered by physiotherapists before and after LT improves the QoL of LT recipients.

1.2. Secondary Aims:

The secondary aims are to investigate whether a remotely monitored 'home-based exercise and theory-based motivation support programme' delivered by physiotherapists before and after LT improves:

- Surgical complications (comprehensive complication index; CCI)
- Mental wellbeing/health (MCS)
- Physical frailty and fitness (LFI/DASI)

- Pre-LT morbidity (UKELD, MELD-Na, Hospital Admission) and mortality
- Post-LT length of ICU/hospital stay, hospital re-admissions and mortality (30,90-day, 1-year)
- Habitual physical activity levels (Daily time spent in light, moderate and vigorous intensity physical activity)
- The frequency, intensity and duration of exercise ('dose') undertaken
- Adherence to HBEP (*intervention arm only*)
- Perceptions of the health care climate (how need supportive/empowering the physio is)
- Basic psychological need satisfaction (i.e. feelings of autonomy, relatedness, competence)
- Self-determined motivation to exercise

1.3. Study objectives:

The main objectives are to conduct a two-centre clinical trial in which 266 patients on the LT waiting list will be randomised to either a) pre- and post-LT remotely monitored 'home-based exercise and theory-based motivation support programme' delivered by physiotherapists (experimental arm, n=133) or b) a standardised patient advise leaflet (control arm, n=133) in order:

1. To determine the effect of the exercise/motivation programme on the QoL of LT recipients using the SF-36v2 health-related QoL questionnaire.
2. To determine the effect of the exercise/motivation programme on physical frailty and fitness of LT recipients using LFI and DASI.
3. To determine the effect of exercise/motivation programme on the morbidity and mortality of LT recipients by recording changes in:
    a. Pre-LT: UKELD, MELD-Na, hospital admissions, deaths.
    b. Post-LT: Post-LT length of hospital/ICU stay, re-admissions, surgical complications and deaths.
4. To measure the habitual levels of physical activity both before and after LT, using a 'blinded' electronic wrist worn accelerometer and assess the impact of the exercise/motivation programme on levels of physical activity.
5. To assess the 'dose' of exercise (frequency, intensity and duration) achieved with the exercise/motivation programme using 'blinded' electronic wrist worn accelerometer and heart rate monitors.
6. To assess adherence to the exercise programme/advice using a self-reported exercise diary and 'blinded' electronic wrist worn accelerometer and heart rate monitor.
7. To investigate how the theory-based motivation support provided by the physiotherapists, affects the patients: a) motivation to exercise; b) feelings of autonomy, relatedness, competence; and c) adherence to the home-based exercise programme (using three psychological questionnaires)?
8. Mechanistic 'Muscle' Sub-study (n= 100): To investigate the dose-dependent effect of the exercise programme on cardiopulmonary fitness (CPET; 6-minute walk test (6MWT)), muscle biology (muscle ultrasound, biomarkers) and their association with QoL

2. TRIAL DESIGN AND SETTING

2.1. Trial design

ExaLT is a phase 2b, open-label, two-centre RCT to assess the efficacy of a home-based exercise and motivational programme in patients before and after LT.

The study will consist of 4 stages:

Stage		Time
1	Pre-screening/identification, enrolment, randomisation and baseline investigations	1-2 weeks
2	Pre-LT waiting list study intervention up to the day of LT (from visit 1 to 6 or LT)	1 – 48 weeks (variable)
3	Post-LT study intervention for 24 weeks (visits 7 to 9)	24 weeks (fixed) End of Intervention; primary endpoint
4	Follow-up assessment (visit 10)	24 weeks after End of intervention (i.e. 48 weeks post-LT)

Due to the unpredictable nature of the timing of LT, the duration of the study intervention ranges from a minimum of 25 (1 week pre-LT; 24 weeks post-LT) to a maximum of 72 weeks (48 weeks pre-LT; 24 weeks post-LT). The maximum duration of the trial for an individual participant, including screening, intervention and the follow up visit will be approximately 2 years (96 weeks). In the event that a participant is not transplanted after 48 weeks study intervention, the intervention will be terminated and with the participants consent their data will be collected until the trail end date.

Eligible participants will be randomly assigned to one of two groups:

- Group 1: Intervention group. Remotely-monitored home-based exercise and theory-based motivation support programme delivered by the physiotherapists on the LT waiting list (max. 48 weeks) through to 24 weeks post-LT.
- Group 2: Control group. Patient 'exercise' advice leaflet before and after LT.

The interventions will be delivered to the participants in two phases: phase 1 pre-LT (maximum 48 weeks) and phase 2 post-LT (24 weeks). All patients that are transplanted within 48 weeks of randomisation will receive a fixed 6 months of intervention after LT. Group 1 and 2 will contain

approximately the same proportion of age groups, disease severity (UKELD), gender, trial site and 'muscle' sub-study as a result of stratified randomisation.

2.2. Trial setting

The trial will take place across two NHS LT centres in England, namely QEUHB and RFH.

2.3. Mechanistic 'muscle' sub-study (n=100)

The main aim of the optional 'muscle' sub-study is to undertake a detailed evaluation of the biological and physiological mechanisms that may underlie any exercised-induced improvements in clinical outcomes, including QoL and physical function/frailty. A better understanding of how exercise works (i.e. on the muscular and cardiopulmonary systems) will guide future studies in terms of exercise dose-response ('frequency', 'intensity', 'duration') that are required in patients with end-stage liver disease to maximise the efficiency and longevity of LT. The sub-study will aim to recruit 100 participants (approx. 50 in each study arm) and will take place at three time-points: pre-LT visit 1 (baseline, week 0), pre-LT visit 2 (week 6), and at the post-LT visit 9 (24 weeks post-LT; end of intervention). See **Section 15.0** for a more detailed summary of the 'muscle' sub-study.

2.4. Assessment of risk

All clinical trials can be considered to involve an element of risk and in accordance with the Birmingham Clinical Trials Unit (BCTU) SOPs, this trial has been risk assessed to clarify any risks relating uniquely to the ExaLT trial beyond that associated with usual care. A risk assessment has been conducted and concluded that this trial is low risk. An ongoing evaluation of risk will continue throughout the trial.

3. ELIGIBILITY

3.5. Inclusion criteria

To be eligible to participate in the ExaLT Trial, patients must meet <u>all</u> of the following inclusion criteria:

- Adult patients (aged 18 years or over)
- Patients listed for a cadaveric, primary LT at QEUHB or the RFH
- Being an out-patient at the time of baseline trial visit (consent)

3.6. Exclusion criteria

If <u>any</u> of the following apply, the patient will not be eligible to be recruited into the ExaLT Trial:

- Patients listed for LT for any of the following reasons:
    - super-urgent LT (according to the Kings College criteria)
    - multi-organ transplantation (e.g. combined liver and kidney transplant)
    - live-related donor LT

- re-graft LT

- Patients with an inability to safely comply with the exercise intervention due to:
  - severe hepatic encephalopathy (grade 3 or 4; or as judged by the clinical investigators)
  - oxygen-dependent hepato-pulmonary syndrome
- Patients *without* liver failure, including:
  - liver cancer in the absence of cirrhosis
  - polycystic liver disease
  - rare metabolic/genetic conditions (e.g. glycogen storage disorders)
- Refusal or lacks capacity to give informed consent to participate in the trial, at the point of study visit 1 (baseline)[1]

3.7. Eligibility for mechanistic 'muscle' sub-study (n=100)

To be eligible to participate in the 'muscle' sub-study, patients must meet all of the above eligibility criteria (**section 4.1/4.2**), consent for the main ExaLT trial and provide additional written consent for the sub-study.

3.8. Co-enrolment

The Trial Management Group (TMG) will consider requests for co-enrolment into other trials (e.g. donor graft machine perfusion studies) in accordance with best practice recommendations. Prior to co-enrolment being sanctioned, the following will be reviewed: study design and statistical considerations; legal and ethical considerations; biological and scientific rationale; patient considerations and; logistical and organisational issues. For co-enrolment to occur, an agreement will be reached between the respective trials team prior to the patient being considered for inclusion. A log of all patients co-enrolled will be maintained by the ExALT UK Trial Office.

4. RECRUITMENT AND CONSENT

It is the responsibility of the PI (or designated co-investigator as documented on the signature and delegation log) to obtain written informed consent for each participant prior to performing any trial related procedures.

Potential participants will be identified as described in section 5.9, a member of the patient's healthcare team who is independent of the study will inform them of the study to gauge interest in participation.

If the potential participant is interested in taking part and agree to be approached by a member of the research team, a participant information sheet (PIS) will be provided to them. The PI or delegate will

[1] During the course of the trial, some participants may lose capacity because of complications of their liver condition(s), for example hepatic encephalopathy.

ensure that they adequately explain the aim of the trial, the trial intervention, and the anticipated benefits and potential hazards of taking part in the trial to the participant. They will also explain that participation is voluntary and that the participant is free to decide to take part and may withdraw from the trial at any time. The participant will be given sufficient time to read the PIS and to discuss their participation with others outside of the site research team. The participant will be given the opportunity to ask questions before signing and dating the latest version of the informed consent form (ICF). If the participant then expresses an interest in participating in the trial, they will be asked to sign and date the latest version of the ICF.

The PI or delegate will then sign and date the ICF. A copy of the ICF will be given to the participant, a copy will be filed in the medical notes and the original placed in the investigator site file (ISF). Once the participant is entered into the trial, the participant's trial number will be entered on the ICF maintained in the ISF. In addition, the participant understands and acknowledges that, a copy of the signed ICF will be transferred to the trial team at BCTU for review.

Details of the informed consent discussions will be recorded in the participant's medical notes. This will include date of discussion, the name of the trial, summary of discussion, version number of the PIS given to participant, version number of ICF signed and date consent received. Where consent is obtained on the same day that the trial related assessments are due to start, a note should be made in the medical notes as to what time the consent was obtained and what time the procedures started. For the same process and documentation of consent will be undertaken for participation in the mechanistic 'muscle' sub-study.

It is recognised that some participants may, during the course of the trial, lose capacity because of complications that may occur due to their pre-existing liver condition(s), for example, worsening hepatic encephalopathy. In this situation, we will seek advice from a personal and/or nominated consultee (as per the Mental Capacity Act 2005) as to whether the participant would wish to continue participating in the trial. A personal consultee can be defined as someone who is:

- Engaged in caring for the participant (not professionally or for payment) or is interested in his/her welfare, and
- Is prepared to be consulted

For the reason that family and/or social support (i.e. established friend) is a fundamental requirement during out-patient assessment for elective LT, it is extremely rare that a personal consultee cannot be found in this setting. To aid in this process, during the informed consent discussion with the participant, we will ask them to identify someone (who fulfils the above criteria), who would be willing to act as a 'personal consultee.' However, in the event a personal consultee cannot be identified, there will be the option to seek advice from a 'nominated consultee.' A 'nominated consultee' includes healthcare workers with no involvement in the trial (i.e. medical consultant, paid carer).

Where it is necessary to seek the advice of a consultee, the PI or delegate will ensure that they adequately explain the aim of the trial, the trial intervention, and the anticipated benefits and potential hazards of taking part in the trial to them. They will also explain that participation is voluntary and that they may advise that the participant be withdrawn from the trial at any time. The consultee will be

given sufficient time to read the personal consultee information sheet. The consultee will be given the opportunity to ask questions before signing and dating the latest version of the consultee declaration form. The PI or delegate will then sign and date the consultee declaration form. A copy of the consultee declaration form will be given to the consultee, a copy will be filed in the medical notes and the original placed in the ISF. In addition, the participant understands and acknowledges that, a copy of the signed consultee declaration form will be transferred to the ExaLT Trial Office at BCTU for review.

Should the participant regain capacity, their wishes will supersede those of the consultee.

At each visit, the participant's willingness to continue in the trial will be ascertained and documented in the medical notes. Where the participant lacks capacity, advice will be sought from a consultee as described above. Throughout the trial, the participant (or their consultee) will have the opportunity to ask questions about the trial.

Any new information that may be relevant to the participant's continued participation will be provided. Where new information becomes available which may affect the participants' decision to continue, participants (or their consultee) will be given time to consider and if happy to continue they will be re-consented. Re-consent will be documented in the medical notes. The participant's right to withdraw from the trial will remain.

Electronic copies of the PIS and ICF will be available from the ExaLT Trial Office. The research site is required to present the documents on headed paper of the local institution.

5. ENROLMENT, RANDOMISATION and BLINDING

Potential trial participants will be recruited from the LT services at the supra-regional LT units in QEUHB and RFH.

5.9. Participant Identification and pre-screening:

Patients who are potentially eligible for the trial (**Section 4.0 eligibility criteria**) will be identified by the MDT healthcare professionals (e.g. Hepatologist, Transplant Coordinator, Anaesthetist, Nurses, AHPs) who are directly involved in the patient's routine clinical NHS care – using the following:

- LT waiting list:
    - all patients 'active' on the UK LT waiting list are registered with NHSBT and recorded in a 'live' national database.
    - healthcare professionals (QEUHB and RFH) directly involved with the patients care on the LT waiting list have access to the NHSBT LT registry and their units LT waiting list database (on secure, password protected NHS computers).
    - in addition, both QEUHB and RFH have dedicated LT waiting MDT clinics, which are led by MJA (ExaLT CI) and CM (ExaLT PI RFH), respectively. In these weekly clinics, in which patients on the LT waiting list are under close follow-up, potential participants will

have the trial explained with oral and written information. At this stage the potential trial patient will have the opportunity to ask questions.
    - at the start of the trial, all eligible patients on the LT waiting list at QEUHB and RFH will receive a letter (in the post), which will include a brief overview of the trial and the contact details of the trials team if they would like to receive further information about the trial (i.e. PIS to be posted out).
- Out-patient LT assessment:
    - in total, QEUHB and RFLH undertake 500-600 LT assessments per year
    - during the out-patient LT assessment, patients and their NOK/friend (personal consultee) undergo oral, written and visual education regarding LT (i.e. waiting list, LT, risks, medications, aftercare, research opportunities etc). An overview of the ExaLT trial will be incorporated into the assessment process.
    - Trial information will also be available in the following formats and accessible to patients/public via the BCTU website:
        - ExaLT trial webpage (link to PIS, overview of study, eligibility, trial team contacts, frequently asked questions)
        - ExaLT Patient information leaflet (i.e. pamphlet) will be available in the LT waiting list and assessment outpatient clinics.
        - potential trial participants will then be able to approach the trial teams for further information (i.e. PIS), if they have not already received it via the above.

All identified potential trial participants will either by given the PIS:

- in person at either the liver transplant assessment or in their dedicated liver transplant waiting list clinic/specialist liver clinic (i.e. PSC, HCC)
  and/or
- via post, especially in light of the emergence of telephone/virtual video clinics (as a result of the COVID pandemic)
- after receiving the PIS the potential participant will require greater than 24 hours to read the PIS information and discuss potential participation with friends and family (in particular a personal consultee), prior to providing consent for the trial.

Details of all patients approached about the trial will be recorded on the ExaLT participant screening/enrolment log which will be kept in the ISF, and should be available to be sent to the ExaLT Trial Office upon request.

If the potential trial participant provisionally agrees to enrol in the trial, after reading the PIS information and discussing their potential participation with friends and family (in particular a 'personal consultee'), **a baseline trial visit (visit 1) will be arranged**.

5.10. Enrolment (Trial Entry)

Enrolment to the ExaLT trial (+/- the 'muscle' sub-study) will take place at the baseline trial visit (**Visit 1**). The study team will aim to coincide Visit 1 with the patients next LT waiting list clinic appointment,

to avoid the additional burden of travelling to the LT unit. If this is not possible, the next available date (Monday to Friday) will be arranged.

Consent (see section 5.0)

- NO trial specific examinations, investigations or treatments, that do not involve part of the patient's routine standard healthcare, will be performed prior to obtaining written consent of the patient**.**
- A member of trials team (i.e. CI/PI or designated co-investigator as documented on the signature and delegation log) will discuss with patient all the relevant information, including aims, methods, risk and benefits of the trial, prior to obtaining consent.
- At this stage the patient will also nominate a 'personal consultee' in the event that they lack capacity at any stage of the trial.
- Once valid informed consent (i.e. ICF signed and dated by the patient) the eligibility checklist will be completed

Confirmation of Eligibility

The following will be verified by the research nurse or another clinical member of the trials team:

- Complete patient consent form (including the personal consultee consent)
- Confirmation of all of the inclusion criteria:
    - adult patients (aged 18 years or over)
    - patients listed for a cadaveric, primary LT at QEUHB or the RFH
    - being an out-patient at the time of baseline trial visit
- Review of the exclusion criteria (see **section 4.0**)

5.11. Randomisation

Randomisation will be provided by BCTU using a secure online system, REDCap, thereby ensuring allocation concealment. Unique log-in usernames and passwords will be provided to those who wish to use the online system and who have been delegated the role of randomising participants into the trial as detailed on the ExaLT site signature and delegation log. These unique log-in details must not be shared with other staff and in no circumstances should staff at sites access either system using another person's login details. The online system will be available 24 hours a day, 7 days a week, apart from short periods of scheduled maintenance. In the event that the online system is available, researchers should contact the ExaLT Trial Office.

5.11.1. Randomisation and registration (delete as applicable) process

After eligibility for randomisation has been confirmed and informed consent has been given, the participant will be randomised using the online system. Randomisation forms will be provided to investigators and will be used to collate the necessary information prior to randomisation. All questions and data items on the online randomisation form must be answered prior to a potential participant being randomised into the trial and a unique trial number being issued.

Following randomisation, a confirmatory e-mail will be sent to the local PI and designated members of the trial study team (e.g. local research nurse). The local research team should add the participant to the ExaLT participant recruitment and identification log, which links participants with their unique trial identification number. PIs must maintain this document securely and it must not be submitted to the ExALT trial office. The ExaLT participant recruitment and identification log should be held in strict confidence.

5.11.2. Randomisation method

Randomisation will be provided by a computer-generated programme. Participants will be randomised on a 1:1 ratio to either:

- Group 1: Intervention group. Remotely-monitored home-based exercise and theory-based motivation support programme delivered by the physiotherapists on the LT waiting list (max. 48 weeks) through to 24 weeks post-LT.
- Group 2: Control group. Patient exercise advice leaflet before and after LT.

A minimisation algorithm will be used within the randomisation system to ensure balance in the allocation over the following variables:

- Gender (Male, Female)
- Age (≤55 years, >55 years)
- UKELD score (≤54, >54)
- Trial centre (QEBH, RFH)
- Enrolled in the 'muscle' sub-study (Yes, No)
    - this criteria will become a default "No" for all randomised patients once the target sample size of 100 patients enrolled in the sub-study is reached.

A 'random element' will be included in the minimisation algorithm, so that each participant has a probability (unspecified here), of being randomised to the opposite treatment that they would have otherwise received.

5.12. Blinding

The ExaLT trial is an open-label study. Due to the nature of the study intervention (i.e. exercise and motivation programme) and the fact it is delivered by the study physiotherapists it is not possible to blind the participant or the co-investigators from the allocated study intervention. Importantly, however, the participants will be 'blinded' to the electronic wrist worn accelerometers (and heart rate

monitors) to ensure they are not getting any objective positive or negative feedback from the accelerometers on their physical exertion or activity during the trial. In addition, data analysis of the 'muscle' sub-study will be blinded, in that the individual(s) performing the analysis of the CPET, muscle ultrasound and specialist biomarkers will blinded to the study order of the investigations and allocation of the study intervention; thereby avoiding interpretation bias.

5.13. Informing the participant's General Practitioner (GP)

If the participant has agreed, the participant's GP will be notified that they are in the ExaLT trial, using the approved ExaLT GP letter.

6. TRIAL INTERVENTION

6.1. Overview of Trial intervention (Intervention arm)

The trial intervention will be delivered to participants in two phases:

Phase 1 - Pre-LT: From enrolment into the study (baseline) up to LT. Duration of phase 1 will range from 1 to 48 weeks, due to the unpredictable nature of the timing of LT.

Phase 2 - Post-LT: From day 1 admission to the ward (i.e. discharge from ICU) to 24 weeks post LT. Duration of phase 2 will be fixed at 24 weeks (minus ICU length of stay, median 2-3 days [NHSBT data 2021])

The intervention for both phases will be delivered by study physiotherapists and will comprise of two core components:

1. A remotely-monitored personalised **home-based exercise programme (HBEP)** and
2. **An autonomous motivation enhancement programme, known as *Empowering Physio***, delivered to physiotherapists to support them in delivering the HBEP.

Home-based exercise programme (HBEP) - Following an initial assessment at Visit 1 (see section 7.2), the patients will be provided with a HBEP consisting of five sessions of aerobic and resistance exercise per week. Thereafter, in the pre-LT phase 1, participants will attend up to four face-to-face visits with the physiotherapist (visit 2 - week 6; visit 3 – week 12; visit 4 – week 24; visit 5 - week 36), during which the participant will be assessed and the HBEP revised accordingly. If the participant has not had their LT by week 48 (visit 6) of phase 1, the participant will be withdrawn from the physiotherapist-delivered HBEP (study intervention). After LT, the participant will initially undergo physiotherapist delivered walking and basic exercise programmes (supported in concordance with *Empowering Physio* principles) until discharge from hospital. The HBEP re-commences on discharge from hospital and will be adapted according to the patient's LFI and DASI, performed within 24 hours of expected discharge. The PI and the consultant transplant surgeon will be consulted prior to commencing the HBEP if there are any ongoing surgical complications (i.e. biliary drain in-situ; wound dehiscence etc). After discharge, the participant will have two face-to-face visits (visit 7 - weeks; visit 8 - week 12) with the

physiotherapist. In addition, physiotherapist support to the participants will be provided in the form of virtual or telephone health calls (Telecalls) to allow revisions to their personalised HBEP and the continuing employment of *Empowering Physio* strategies techniques as required. Telecalls will take place in the pre-LT phase 1 at weeks 2, 4, 8, 10, 16 and 20 (pre-LT) and in the post-LT phase 2 at weeks 4, 8 and 10. Section 7.3 and 7.4 provide further detail of the face-to-face visits and the telecalls.

Autonomous motivation enhancement programme - The bespoke *Empowering Physio* programme will be used to equip physiotherapists (see **section 7.2**) with the understanding and skills to support each patient's sense of autonomy, competence and relatedness in delivering the HBEP; in order to help foster more autonomous motivation for uptake and adherence to the HBEP and engagement in exercise overall.

6.2. Physiotherapy Training

To ensure consistency across sites, the physiotherapists will receive formal training from Mrs Felicity Williams (Liver/LT Specialist Physiotherapist; PI) on all aspects of the assessment (including LFI and DASI) and HBEP intervention prior to commencement of the study. Furthermore, the physiotherapists will be trained in the principles and strategies of *Empowering Physio* by Professor Joan Duda (Professor of sport and exercise psychology; co-investigator). The face-to-face training will take place over a 2-3 day structured course (**Table 1**). The overarching aim of the bespoke *Empowering Physio* training programme is to:

1. Enhance physiotherapists' understanding of: (a) what is optimal motivation for exercise and behaviour change; (b) the importance of the motivational 'treatment' climate they create; and (c) how that created climate (the physiotherapists' behaviours) influences patients' motivation for pursuing their physical activity goals and associated well-being.

2. Provide the opportunity for the physiotherapists to: (a) learn what are the 'building blocks' of creating a more empowering motivational treatment climate when working with patients, and (b) develop strategies which facilitate the realisation of these 'building blocks.'

The three day training will involve presentation content and interactive activities to highlight how physiotherapists interact with and provide information and feedback to patients and the implications of such for patients' motivation to engage in physical activity. Physiotherapists will be asked to reflect on their own experiences in clinical practice in regard to optimal and questionable motivational stategies. The workshop will also address the importance of communication style, and 'how ' to exchange with patients so that they feel a greater sense of autonomy, competence and connection in regard to their HBEP. The persuading and directing way of communicating will be contrasted with an evoking, guiding and following manner of exchanging with patients. The physiotherapists will then have the opportunity to identify barriers to creating a more empowering treatment climate and develop potential strategies to overcome this.

Table 1: Study Physiotherapist Training Course

Training Components	Day 1	Day 2	Day 3
Study logistics	√		
Functional and nutritional assessments (LFI/6MWT/MAMC)	√		
Questionnaires (DASI/PCS-SF-36v2/MCS-SF-36v2/HCCQ/PNES/BREQ-2)	√		
Aerobic and Resistance exercise theory	√		
Practical exercises	√		
Patient education package	√		
Muscle Ultrasound	√		
Principles and strategies of *Empowering Physio*		√	
Practical application of *Empowering Physio*			√
Principles and strategies to delivery of face-to-face consultations, patient education session and Telecalls			√
Total time (hours)	7	7	7

The principles and embedded strategies to more empowering physiotherapy will be revisited the following day and reviewed to ensure understanding and application. The physiotherapists will then have the opportunity to consider the face-to-face consultations they will have with their patients (with particular emphasis on the initial participant education session, exercise familiarisation, and provision of the written exercise programme) *and* Telecalls and develop/'bring to life' a planned approach (i.e. specify motivational aims, strategies) to make these exchanges more empowering. Role playing will be used to exemplify the empowering strategies that the physiotherapists will employ and address challenges that may arise.

6.2.1. Fidelity testing of physiotherapist-delivered intervention

The implementation fidelity of the physiotherapist-delivered intervention will be assessed in regard to (1) expected content conveyed (e.g. explanation and demonstration of the HBEP to the patient), and (2) the degree to which the behaviours of the physiotherapist (when interacting with the patient) were motivationally empowering (and thus supportive of the patient's autonomous motivation for exercise). The interactions between physiotherapist and patient will be examined (using audio for telecalls and visual recordings for face-to-face visits) in the case of two physiotherapists (randomly selected at each trial site) in regard to observation of the following sessions involving one consented patient:

- The baseline visit 1 (week 0 pre-LT) session including exercise training/education
- One telecall follow-up (either weeks 2, 4 or 8)
- One pre-LT face-to-face follow-up visit (either visit 2 or 3)
- One post-LT face-to-face follow-up visit (either visit 7 or 8)

A modified (for the present exercise intervention content) of the Interpersonal Support in Physical Activity Consultations Observational Tool (ISPACOT) (399) will be employed to evaluate the degree to which the physiotherapists conveyed the expected information, as intended in the face-to-face

consultations and telecalls *and* the motivational climate manifested during these treatment sessions. In regard to the latter, the ISPACOT assesses four aspects of the treatment climate: the degree to which the physiotherapist is autonomy supportive, demonstrated social support/caring, provided structure, and exhibited interpersonal control.

6.3. Phase 1: Pre-LT trial intervention

Pre-LT Phase 1 of the HBEP will commence the day after baseline visit 1 (maximum 3 days post visit 1) and end on either a) the day of LT, or b) after 48 weeks (visit 6) if LT has not taken place. Details of the intervention timeline are summarised in **Table 2**.

Table 2: Study Intervention timelines. Key: *A/R = aerobic/resistance; HR = heart rate; LT = liver transplant; V = visit; THC = virtual/telephone health call; W = week*

	PHASE 1 TRIAL INTERVENTION												PHASE 2 TRIAL INTERVENTION									
Trial Visits **Trial Intervention**	**V1** **W0**	THC W2&4	Devices W4-6	**V2** **W6**	THC W8&10	Devices W10-12	**V3** **W12**	THC W16&20	**V4** **W24**	**V5** **W36**	**V6** **W48**	**LT**	**IP** **stay**	THC W4	Devices W4-6	**V7** **W6**	THC W8&16	Devices W10-12	**V8** **W12**	Devices W22-24	**V9** **W24**	**V10** **W48**
Education																						
Patient education session	**X**																					
Devices and Handouts																						
Accelerometer	**X**		X			X									X			X		X		
HR monitor (during structured exercise session only)	**X**		X			X									X			X		X		
Participant Diary issued	**X**												**X**									
Exercise instruction																						
Pre-LT A/R exercise plan	**X**																					
Review and adaptation of A/R exercises		X		**X**	X		**X**	X	**X**	**X**			**X**	X		**X**	X		**X**			
Review of participant diary				**X**			**X**		**X**	**X**	**X**					**X**			**X**		**X**	**X**
Post-LT A/R exercise plan													**X**									
Empowering Physio																						
Identify knowledge about benefits of exercise	**X**																					
Link exercise to personally meaningful goals/events	**X**																					
Decisional balance patient centred goal setting	**X**	X		**X**	X		**X**	X	**X**	**X**	**X**			X		**X**	X		**X**		**X**	
Supports attempts to change behaviour	**X**	X		**X**	X		**X**	X	**X**	**X**	**X**			X		**X**	X		**X**		**X**	
Normalise failed attempts		X		**X**	X		**X**	X	**X**	**X**	**X**			X		**X**	X		**X**		**X**	
Problem solving		X		**X**	X		**X**	X	**X**	**X**	**X**			X		**X**	X		**X**		**X**	

6.3.2. Visit 1 (day 0) – Design and education of personalised HBEP

After obtaining consent and completion of baseline assessments (see section 9.0), participants will meet the study physiotherapist. The baseline assessments, along with *Empowering Physio* strategies, will be used to design a personalised written HBEP for the participant. Baseline LFI and DASI will be used to guide the entry level of difficulty for the aerobic and resistance-based exercises, respectively (**Tables 3 and 4**). In addition, the entry level of difficulty for the exercises will also be influenced by discussions with the participant on ways to employ strategies to support autonomous motivation for exercise adoption and engagement. The participants will then attend a physiotherapist-delivered training session (group session; maximum four participants/day), which will consist of: a) patient education (1 hour), b) exercise familiarisation (1 hour) and c) issuing of devices and written information (1 hour). Details of which are provided in **Table 3**.

- *A) Patient education:* Patient education sessions on the topics of general benefits of exercise, breathless management, pacing, rate of perceived exertion and nutrition pre-and post-exercise, will be delivered in the format of power point presentation and informal discussion by the physiotherapist. In accordance with *Empowering Physio* principles, the education sessions will be delivered in a manner that makes it more likely that the information conveyed is personally meaningful and confidence enhancing. Therefore, being more likely to increase feelings of autonomy, competence and relatedness towards exercise by the participant.

- *B) Exercise familiarisation:* Participants will be taught a series of body-weight resistance exercises performed in a circuit (**Table 4**). The aim of this session is to familiarise the participant with the exercises and the use of the rate of perceived exertion tool for monitoring exercise intensity. This session will also provide an opportunity for the physiotherapist to ensure correct and safe techniques. Participants will have the opportunity to voice any concerns they have regarding the exercises and allow for these concerns to be resolved prior to completing the exercises at home. In line with *Empowering Physio* principles, the physiotherapists will also provide responsive, meaningful feedback to ensure participant's individual needs are met. For example, the physiotherapist will be guided by the participant and adjust exercise levels as needed to ensure participants feel competent in their exercise efforts. Throughout the session, physiotherapists will acknowledge effort and progress.

- *C) Written information:* Using the results of the baseline assessments of LFI and DASI (**Figures 1 and 2**) and the discussions had with the participant, the physiotherapist will provide a personalised written aerobic and resistance HBEP. The details of which can be found below. Furthermore, the participant will be provided with a participant diary to record their completed exercise sessions.

Level of difficulty	Exercise	Intensity	Duration (mins)
1	Walking/cycling/swimming/cross-trainer/running	1x5mins @ RPE 12-14 1x3mins recovery @ RPE 9-11 1x5mins @ RPE 12-14	13
2	Walking/cycling/swimming/cross-trainer/running	1x7mins @ RPE 12-14 1x3mins recovery @ RPE 9-11 1x7mins @ RPE 12-14	17
3	Walking/cycling/swimming/cross-trainer/running	1x10mins @ RPE 12-14 1x3mins recovery @ RPE 9-11 1x10mins @ RPE 12-14	23
4	Walking/cycling/swimming/cross-trainer/running	1x12mins @ RPE 12-14 1x3mins recovery @ RPE 9-11 1x12mins @ RPE 12-14	27
5	Walking/cycling/swimming/cross-trainer/running	1x10mins @ RPE 12-14 1x3mins recovery @ RPE 9-11 1x10mins @ RPE 12-14 1x3mins recovery @ RPE 9-11 1x10mins @ RPE 12-14	33
6	Walking/cycling/swimming/cross-trainer/running	1x15mins @ RPE 12-14 1x3mins recovery @ RPE 9-11 1x15mins @ RPE 12-14	33
7	Walking/cycling/swimming/cross-trainer/running	1x20mins @ RPE 12-14 1x3mins recovery @ RPE 9-11 1x10mins @ RPE 12-14	33
8	Walking/cycling/swimming/cross-trainer/running	1x30mins @ RPE 12-14	30
9	Walking/cycling/swimming/cross-trainer/running	1x35mins @ RPE 12-14	35
10	Walking/cycling/swimming/cross-trainer/running	1x40mins @ RPE 12-14	40

Table 3: Aerobic exercise programme

6.3.3. Aerobic exercise component of the HBEP

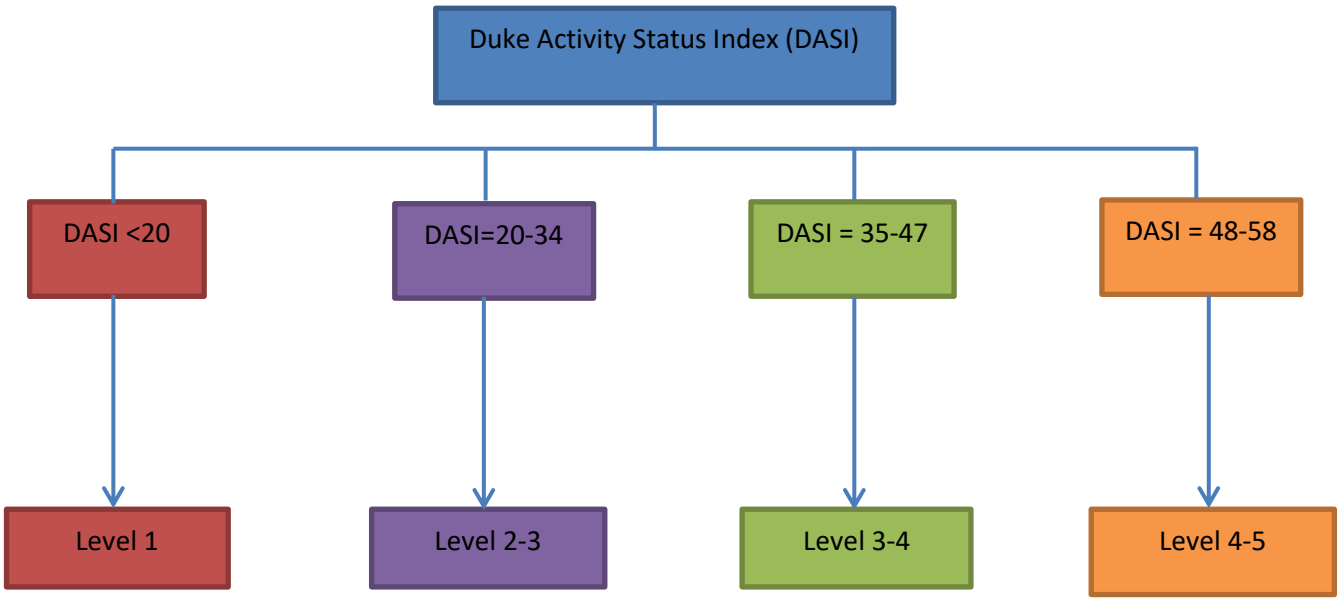
The initial level (duration, recovery period, intensity) of aerobic exercise sessions will be determined from the baseline DASI (**Figure 1**), while accounting for any exercise limiting comorbidities, such as ascites, peripheral oedema and/or hepatic encephalopathy. It will be recommended to the participants

that they aim to complete two sessions of aerobic exercise per week. In line with *Empowering Physio* theory, a rationale for this recommendation will be provided. The participants will also be asked to select their exercise modality of choice from the following options; walking, cycling, swimming, cross-trainer, rowing ergo or running. In collaboration with the physiotherapist, the participant will be able to change their choice of modality week by week or continue with the same choice depending on their preferences. Furthermore, the physiotherapist will involve the participant in discussions about previous positive exercise experiences to facilitate personal goal setting. The level set will be appropriate to the participant's current level of function but also ensure the participant feels competent in their exercise effort. Each aerobic session will consist of alternating "work" and "active rest" periods:

- During the work periods, participants will be asked to exercise at a moderate intensity (rate of perceived exertion (RPE) score of 12-14 (6-20 scale).
- During their active rest periods, participants will be asked to work to a RPE of 9-11. Details of the aerobic exercise intervention and levels of difficulty are detailed in **Table 3**.

Exercise intensity will be progressed depending on the feedback from regular Telecalls to the participant (weeks 2, 4, 8, 10, 16 and 20).

Figure 1 - Flow diagram for use of DASI when prescribing the 'entry level' aerobic exercise programme



Participants will be asked to participate in a 20 minute circuit of bodyweight resistance exercises twice weekly on alternate days to the aerobic sessions. The circuit will consist of four cycles of 8-12 repetitions of five exercises, chosen by the patient (**Table 4 and 5**) with two minutes of "active rest" (walking slowly on the spot) between each exercise and each cycle. The programme and entry level will be developed according to baseline LFI (**Figure 2**), and a trial of 8-12 repetitions exercises within the designated entry level. Furthermore, the entry level will be discussed collaboratively with the participant to support feelings of competence and autonomy.

The participant will be instructed to terminate each set of an exercise when they reach a "repetitions in reserve" (RIR) of 1-2; that is, they feel they could complete 1 or 2 additional repetitions, but no more. The participant will be advised to progress to each level of difficulty once they can achieve 12 repetitions with 1-2 RIR and depending on feedback from the Telecalls at weeks 2, 4, 8, 10, 16 and 20. Details of the resistance exercise circuits and levels of difficulty are detailed in **Table 4 and 5**.

Figure 2 - Flow diagram for use of LFI when prescribing the 'entry level' resistance exercise programme

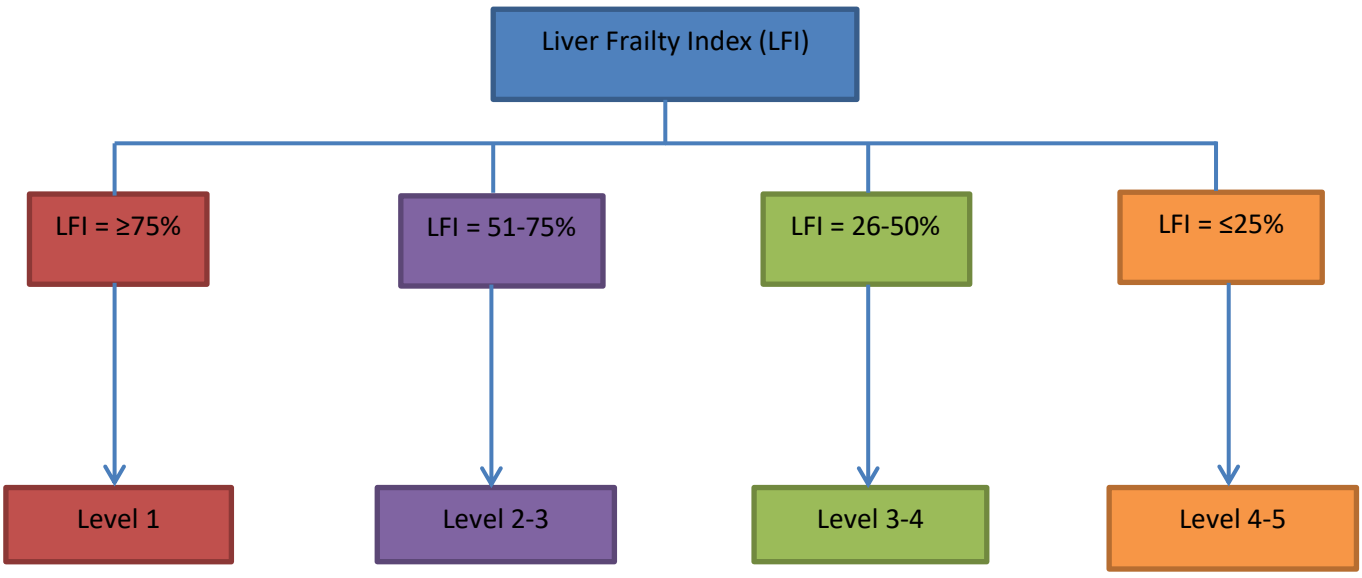


Table 4 - Resistance Exercises

Muscle Group	Exercise	1	2	3	4	5	6
Upper Limb Press	**Horizontal press**	Wall press-up	Press-up on knees	Hands-elevated press-up	Progressively lower hands-elevated press-up	Press-up	Press-up with progressive band resistance
	Vertical press	Overhead press, arms only	Overhead press with light weight (e.g. soup cans)	Overhead press with heavier weight (e.g. water bottles)	Pike push-up, hands on raised surface	Pike push up	Pike push-up, feet elevated to knee height
Upper Limb Pull	**Horizontal pull**	Two-arm row with light weight (e.g. soup can)	One-arm row with light weight (e.g. soup cans)	Two-arm row with heavier weight (e.g. water bottles)	One-arm row with heavier weight (e.g. water bottles)	Two-arm row with progressive band resistance	One-arm row with progressive band resistance
	Lateral/Vertical pull	Lateral rotation with yellow TB	Bilateral abduction with TB	Diagonal TB pull	Vertical pull down with yellow TB	Vertical pull down with red TB	Vertical pull down with green TB
Lower Limb	**Squat**	Raised surface chair stands	Wall squat	Chair stands	Full squat	Squat with light weight (e.g. soup cans)	Squat with progressive band resistance
	Lunge	Static lunge with support	Static lunge without support	Dynamic half lunge	Dynamic full lunge	Walking lunge	Walking lunge with progressive load
	Step ups	Low step-up (e.g. 1 stair)	Low step-up with knee raise	Low step-up with knee raise and light weight (e.g. soup cans)	High step-up with high knee	High step-up with high knee and light weight (e.g. soup cans)	High weighted step up with high knee and progressive load
Core Stability	**Anti-anterior flexion**	Four-point kneeling holds	Four-point kneeling with leg raises	Four point kneeling alternate arm and leg raises	Kneeling plank	Plank	Plank with progressive load
	Glute med/anti-lateral flexion	Clams	Clams heels raised	Straight leg clam	Elbows-elevated side plank	Elevated side plank	Side plank
	Extension	Pelvic tilt in crook lying	Bridges	Bridges with yellow TB	Bridges with red TB	Bridges with red TB and heel raise	Bridges with red TB straight leg reps

Table 5 – Resistance Exercise Session Template

Nº of exercises	Nº of circuits	Repetitions	Rest period between circuits (mins)	Total time (mins)
1x upper limb push 1x upper limb pull 2x lower limb 1x core/balance	4	8-12	2	26

6.3.5. Virtual or telephone health calls ('Telecalls')

At weeks 2, 4, 8, 10, 16 and 20, the participant will receive either a virtual or telephone health call by the physiotherapist, known as a 'Telecall'. The purpose of these calls (duration 15-30 minutes) will be to:

- Identify any adverse events or areas of concern
- Gain feedback from the participant regarding the HBEP
- Provide motivational support for engagement with the HBEP through the implementation of *Empowering Physio* strategies. For example, to empower patients in their attempts to be active, the physiotherapists will support attempts to change behaviour, problem solve and to develop strategies to overcome personally-reported barriers and enhance self-efficacy for exercise. It is also an opportunity to revisit goals to ensure they are aligned with participant's perceptions of their exercise competencies (**Table 2**).
- Guide weekly progression of exercises and goal setting.

An interview guide underpinned by *Empowering Physio* principles and related motivation-based theories of behaviour change, will be used to provide a standardised framework of the Telecalls at both sites.

6.3.6. Face-to-face clinic visits (visits 2, 3, 4, and 5)

Participants will attend the hospital (QEUHB or RFH), in line with their routine waiting list clinic appointment (*where possible*), at weeks 6 (+/- 3 days), 12 (+/- 7 days), 24 (+/- 7 days) and 36 (+/- 7 days). At these visits, a repeat of the baseline assessment, including LFI and DASI, will be undertaken (see Procedure section 9.0). The results of these assessments, review of the participant exercise diary and discussions with the participant themselves will be used to progress exercises and revise goals of their HBEP. As per *Empowering Physio* principles strategies, the active role of the participant in the decision-making process, regarding progression and goal revision, will continue to support more autonomous reasons for engagement in the HBEP. Revisions will be based upon physical frailty/function scores (LFI, DASI) and the participant's owned perceived progress with the training HBEP. Of note, visit 6 (48 weeks +/- 14 days) will include a repeat of the baseline assessments and will mark the end of the study intervention (HBEP, motivation programme) if the participant has undergone LT.

6.4. Phase 2: Post-LT trial intervention

Post-LT Phase 2 of the HBEP will commence on day 1 of admission to the post-LT ward (i.e. within 24 hours of discharge from ICU) and end 24 weeks after the date of the LT surgery (visit 9). Details of the intervention timeline are summarised in **Table 2**.

6.4.7. Day 1 of ward admission (i.e. discharge from ICU) to discharge from hospital post-LT

The trial physiotherapists will review the participant on the post-LT ward, within 48 hours of discharge from ICU. The participants will start a supervised progressive walking programme, based upon the participant's current level of physical frailty/function, in keeping with post-surgical care. If able, the participant will be asked to complete a walk (distance determined by physiotherapist, based upon participant's current level of function) twice daily working to a RPE of 12-14 throughout the walk. Distance walked should be increased on a daily basis provided the participant is medically safe to achieve this, and feels competent in doing so. In addition, the participant will also be asked to complete twice daily a basic exercise programme consisting of upper limb, lower limb, balance, coordination and core-strengthening exercises (**Table 6**). This post-LT exercise 'inpatient' programme will also be supported in concordance with *Empowering Physio* principles. If there are concerns by the physiotherapist about the patient's safety to exercise (i.e. walk, chair stand etc), the patients consultant (i.e. surgeon, hepatologist) and clinical team (i.e. nurse) will be consulted, as per routine NHS care.

Within 48 hours of discharge, a repeat of the baseline assessments, including LFI and DASI, will be undertaken on the ward (see Procedure **section 9.0**). The results of these assessments, along with *Empowering Physio* strategies, will be used to prescribe a personalised written HBEP for the participant post-LT. The participant will also be given participant exercise diary.

Table 6: Post-Liver Transplant Basic Exercise Programme

Level	Exercise	Sets/Reps
1	Marching on the spot Pelvic tilts Wall squat Wall press	3x8-12reps
2	Step-ups Bridge holds (5 seconds) Chair stands Arm raises	3x8-12reps
3	Step-up high knees Single leg bridge holds (5 seconds) Squats Arm raise with Theraband©	3x8-12reps

6.4.8. Face-to-face clinic visits (visits 7 and 8)

Participants will attend the hospital (QEUHB or RFH), in line with their routine post-LT follow-up clinic appointment (*where possible*), at weeks 6 (visit 7; +/- 7 days) and 12 (visit 8; +/- 7 days). At these visits, a repeat of the baseline assessment, including LFI and DASI, will be undertaken (see Procedure section 9.0). As per phase one, the physiotherapist will use these assessments along with *Empowering Physio* techniques and the participant's owned perceived progress to revise their personalised HBEP. Of note, visit 9 (24 weeks post-LT +/- 7 days) will mark the end of the study intervention (HBEP, motivation programme).

6.4.9. Virtual or telephone health calls ('Telecalls')

Participants will receive a 'Telecall' (duration 15-30 minutes) by the physiotherapist at weeks 4, 8, and 10 post-LT. The Telecalls will follow the same format as phase one with the purpose to highlight any participant concerns or adverse events, as well as to gain feedback on the exercise intervention. However, the *Empowering Physio* delivery will now shift to employ strategies for the participant to foster long-term autonomous motivation and maintenance of exercise behaviour.

6.5. Control (comparator) arm

The control arm will be delivered during the pre-LT (phase 1) and post-LT (phase 2) phases of the trial. Participants will receive a standardised patient information 'exercise' leaflet (**Appendix 1 and 2**), which includes standard written advice on physical activity and exercise before and after LT.

6.5.10. Phase 1 - Visit 1 (day 0) – control arm

Following baseline assessment (see procedures 9.0) participants will receive a 20 minute face-to-face consultation with the physiotherapist, during which they will receive verbal and written (patient leaflet) advice on the generic benefits of exercise pre-LT. This will include information on how to maintain physical activity and exercise levels whilst on the LT waiting list, as well as four basic resistance exercises for participants to complete (as described in the leaflet). As part of this consultation, the physiotherapist will demonstrate these exercises and practice them with the participant to ensure they are safe to complete at home. As per the intervention arm, the participants will provided with a participant diary to record an exercise they perform throughout phase 1.

6.5.11. Phase 1 - Face-to-face clinic visits (visits 2, 3, 4, and 5)

Participants will attend the hospital (QEUHB or RFH), in line with their routine waiting list clinic appointment (*where possible*), at weeks 6 (+/- 3 days), 12 (+/- 7 days), 24 (+/- 7 days) and 36 (+/- 7 days). At each visit, the participant will have an opportunity to discuss any concerns regarding physical activity or exercise they have with the physiotherapist (15 minutes). However, the physiotherapist will only provide information in line with established generic physical activity and exercise guidelines on the patient exercise leaflet. Furthermore, the participant will not receive any telecalls during phase 1 of the study. Of note, visit 6 (48 weeks +/- 14 days) will mark the end of control arm (standardised patient advice leaflet) if the participant has undergone LT.

6.5.12. Phase 2 – post LT Day 1 of ward admission to discharge from hospital post-LT

As per the intervention, phase 2 of the control arm will commence on day 1 of admission to the post-LT ward (i.e. within 24 hours of discharge from ICU) and end 24 weeks after the date of the LT surgery (visit 9). The trial physiotherapists will review the participant on the post-LT ward, within 24 hours of discharge from ICU. The participants will start a supervised progressive walking programme, based upon the participant's current level of physical frailty/function, in keeping with routine post-surgical care. If able, the participant will be asked to complete a walk (distance determined by physiotherapist, based upon participant's current level of function) twice daily working to a RPE of 12-14 throughout the walk. Distance walked should be increased on a daily basis provided the participant is medically safe to achieve this, and feels competent in doing so. However, unlike the intervention group, no other formal exercises will be provided for the participant. If there are concerns by the physiotherapist about the patient's safety to exercise (i.e. walk etc), the patient's consultant (i.e. surgeon, hepatologist) and clinical team (i.e. nurse) will be consulted, as per routine NHS care.

Within 48 hours of discharge, the participant will receive a 30-minute inpatient consultation with the physiotherapist where they will be advised to gradually increase their exercise post-LT. This information will be supported with the phase 2 post-LT patient 'exercise' advice leaflet; which will include four basic resistance exercises. The participant will also be given participant exercise diary to record any formal exercise completed at home.

6.5.13. Phase 2 - Face-to-face clinic visits (visits 7 and 8)

Participants will attend the hospital (QEUHB or RFH), in line with their routine post-LT follow-up clinic appointment (*where possible*), at weeks 6 (visit 7; +/- 7 days) and 12 (visit 8; +/- 7 days). As per phase 1 of the control arm, the physiotherapist will continue providing the advice highlighted in the patient 'exercise' advice leaflet and advise the participant to continue recording any formal exercise sessions in their participant diary. Of note, visit 9 (24 weeks post-LT +/- 7 days) will mark the end of the study of the control arm advice (standardised patient advice leaflet). Furthermore, no Telecalls will be made to the comparator group throughout phase 2 of the control arm.

6.6. Trial intervention modification or discontinuation

6.6.14. Trial intervention modification (unscheduled)

Throughout phases 1 and 2 of the trial intervention the level of HBEP will modified (scheduled) at the face-face trial visits or via the Telecalls, based upon the participants physical frailty/function assessments (LFI, DASI) and the participant's owned perceived progress with the HBEP. In addition, the HBEP will be modified (unscheduled) by the physiotherapist in the event that the participant (or personal consultee), clinician (including GP, local hospital clinical team) and/or a clinical member of the study team highlight that there is:

- There is a significant deterioration in the participants liver disease severity (i.e. severe hepatic encephalopathy, new onset moderate/severe ascites, worsening anaemia, SBP), as judged by the PI/CI or nominated clinical co-investigator on the delegation log.
- There is acute deterioration in the participants health status that does not require hospitalisation, but will impact on the participants ability to comply with the HBEP, as judged by the PI/CI or nominated clinical co-investigator on the delegation log. e.g.
    - musculoskeletal injury
    - systemic illness (i.e. viral illness, urinary tract infection (UTI), pneumonia)
- There is acute deterioration post-LT (specifically) in the participants health status that does not require hospitalisation, but will impact on the participants ability to comply with the HBEP, as judged by the PI/CI or nominated clinical co-investigator on the delegation log. e.g.
    - post-LT surgical complication (e.g. incisional hernia)
    - complications of immunosuppression (e.g. mycophenolate induced diarrhoea/gastrointestinal upset; tacrolimus induced neuropathy/headaches)
    - opportunistic infection (e.g. cytomegalovirus [CMV])

These ad-hoc (unscheduled) modifications to the HBEP will take place via an unscheduled Telecall/face-to-face clinic visit or the next scheduled Telecall/Face-to-face trial visit.

6.6.15. Trial intervention discontinuation (unscheduled)

The trial intervention (HBEP and motivation programme) will be discontinued (scheduled) on the day of LT and until the participant is discharged from ICU to the post-LT surgical ward. The trial intervention will be discontinued (unscheduled) If the participant is re-admitted to ICU during the post-LT period of the trial. The HBEP and motivation program will be restarted as discussed in **section 7.4**.

The trial intervention (HBEP) will be discontinued immediately in the event of any of the following:

- Serious adverse events (SAE; refer to **section 10.0** for definitions). Examples include:
    - fall/musculoskeletal injury (i.e. fracture, head injury)
    - cardiac event or cerebrovascular accident [CVA] (i.e. myocardial infarction/angina, arrhythmia, stroke, transient ischaemic attack, Cerebral haemorrhage)
    - other surgical or medical emergencies (e.g. diabetic ketoacidosis, bowel obstruction, severe anaemia etc)
    - pre-LT (on LT waiting list):
        - hepatorenal syndrome (HRS)/acute kidney injury (AKI)
        - severe hepatic encephalopathy
        - variceal haemorrhage requiring oesopho-gastro-duodenoscopy (OGD) +/- therapy
        - sepsis secondary to spontaneous bacterial peritonitis (SBP) or cholangitis
        - severe jaundice
    - Post-LT:
        - post-LT surgical complication (e.g. bile leak, peritonitis, hepatic artery thrombosis (HAT), wound dehiscence)

- post-LT medical complications (e.g. organ rejection, opportunistic infections e.g. CMV, graft dysfunction, AKI)

In the event that an SAE has resolved, the participant will *only* resume the trial intervention (HBEP) on the advice of clinical members of the trial team (including the PI/CI or nominated member of the research team as per the delegation log) after a clinical review of their health status and physical function. This clinic review will either take place via an unscheduled Telecall/face-to-face clinic visit or at the next scheduled Telecall/Face-to-face trial visit. If there are concerns by the physiotherapist about the patient's safety to perform the HBEP, the patient's consultant (i.e. surgeon, hepatologist) will be consulted as per routine NHS care and the PI (or CI) for the trial site will be informed.

6.7. Adherence to trial intervention (HBEP)

Adherence to the HBEP will be assessed using:

- Self-reported participant 'exercise' diary: The participants will be asked to fill in their diary every time they complete a session of structured exercise (maximum 5 sessions of HBEP per week). The study physiotherapists will be able to monitor adherence to the HBEP by reviewing the diaries at each face-to-face visit and during the scheduled Telecalls with the participant.
- Wrist-worn accelerometers (Actigraph GT9X) and heart rate monitors: The accelerometers will be worn 24 hours/day for set 14 day periods and the heart rate monitors (chest strap) will be warn during structured exercise sessions in the same set 14 day periods (see **sections 8.2 and 9.0**). Accelerometers will be initialised to ensure participants will not receive any feedback on their activity levels during their enrolment in the trial. i.e. accelerometers are being employed as secondary outcome measures, not as part of the intervention. The devices are waterproof and do not need to be removed for bathing, showering or swimming; thereby not affecting the adherence analysis. The physical activity and heart rate raw data (i.e. frequency, intensity, duration) will be collated by the physiotherapist at pre-LT visits 2-4 and post-LT visits 7-9 and safely stored for data analysis.

6.8. Continuation of intervention after completion of the trial

The participant will officially complete the remotely monitored 'home-based exercise and theory-based motivation support programme' delivered by physiotherapists at visit 9 (24 weeks after LT). The hypothesis is that the participant will then have the physical functional status, knowledge, competence, confidence and self-determined motivation to continue to engage with unsupervised exercise in the future. This hypothesis will be assessed at follow-up visit 10 (48 weeks post-LT; 24 weeks after stopping physiotherapy delivered intervention) with measures of physical and mental well-being (PCS and MCS SF-36v2 QoL questionnaire) and behaviours/motivation towards exercise (behavioural/psychological based questionnaires) (see sections 8.0 and 9.0). After the participants have completed the trial at visit 10, they will be followed up in their routine NHS post-LT clinic and will receive the standard of healthcare in place at that time. There will be no possibility of the prescribed physiotherapist delivered 'home-based exercise and theory-based motivation support programme' until the results of the trial are analysed and published.

7. OUTCOME MEASURES

All primary and secondary outcomes measures will be completed at the following time-points (*unless stated*):

- **Pre-LT (phase 1):** baseline visit 1 (0 weeks; pre-intervention), visit 2 (6 weeks +/- 3 days), visit 3 (12 weeks +/- 7 days), visit 4 (24 weeks +/- 7 days), visit 5 (36 weeks +/- 7 days) and visit 6 (48 weeks +/- 7 days). Of note, as the timing of LT is unpredictable, the participant will enter the post-LT phase 2 of the trial on the day of LT, irrespective of how many study visits they completed in pre-LT phase 1.

- **Post-LT (phase 2):** visit 7 (6 weeks post-LT +/- 7 days), visit 8 (12 weeks +/- 7 days), visit 9 (24 weeks +/- 7 days; end of intervention) and visit 10 follow-up (48 weeks +/- 14 days)

7.9. Primary outcome(s)

The primary outcome measure for this trial is the **PCS from the SF-36v2 health-related QoL questionnaire at 6 months (defined as 24 weeks) post LT**. The SF-36v2 questionnaire includes 36 questions composed of eight multi-item scales, which reflect the impact of health problems on both the physical and mental condition of the patient.(400, 401) A higher score reflects better quality of life. Two summary sub-scores can be calculated which are weighted combinations of the 8 scales, one to reflect the impact on physical function (PCS) and one to reflect the impact on psychological function, known as the mental component score (MCS).(402) Justification for the primary outcome measure is summarised in **Section 1.2.4**. Scoring of the SF-36v2 questionnaire will based on the instructions provided in the SF-36v2 user's manual.(403)

7.10. Secondary outcome(s)

The 'Key' secondary outcome measure to be assessed at 6 months (24 weeks) post-LT is:

- **Comprehensive Complications Index (CCI)**: The CCI is a well validated, reproducible tool in surgery and LT, which provides a 0-100 index (0=no complications, 100=death) using the frequency and grade (CTCAE grade) of surgical-related complications (i.e. wound dehiscence, bile leak, abdominal collections, bleeding, hepatic artery thrombosis etc).(404, 405) The sample size will enable an accurate, representative comparison of the intervention and control arms 6 months post-LT to investigate if the HBEP significantly reduces surgical complications post-LT.

The other secondary outcome measure to be assessed at 6-months (24 weeks) post LT are:

- **Mental component score (MCS) of SF-36v2 health-related QoL:** The SF-36v2 questionnaire is a practical, reliable, and valid measure of physical and mental health that can be completed in 5 to 10 minutes. Scoring of the SF-36v2 questionnaire is as described above for PCS SF-36v2 questionnaire.
- **Liver frailty index (LFI):** The LFI is a composite metric of three performance-based measures: hand grip strength (HGS), time to do 5 chair stands (seconds) and time holding 3 balance positions (feet side by side, semi-tandem and tandem) to objectively assess physical frailty in ambulatory patients with end-stage liver disease.(136) The LFI score can be calculated using an on-line calculator (available at: *http://liverfrailtyindex.ucsf.edu*) with patient physical frailty categorised as robust, pre-frail and frail according to their index (index = <3.2 (robust), 3.2-4.5 (pre-frail), >4.5 (frail)). In addition to the time-points listed above, LFI will be completed on the day admission for LT (immediately pre-LT) and prior to discharge (within 48 hours) from hospital post-LT.
- **Duke activity status index (DASI):** The DASI is a 12 item self-reported assessment of functional capacity that requires minimal time to complete.(163) It provides prognostic information in a variety of chronic diseases and can be used as an index of disease progression over time (164-166). In addition to the time-points listed above, DASI will be completed prior to discharge (within 48 hours) from hospital post-LT.
- **Pre-LT morbidity (UKELD, MELD-Na, Hospital Admissions) and mortality:**
    - **UKELD**: is a scoring system (INR, serum bilirubin, creatinine and sodium) which is used to predict the prognosis of patients with end-stage liver disease.(406)
        - UKELD = [(5.395Xin(INR)) + (1.485Xin(creatinine)) + (3.13Xin(bilirubin)) - (81.565Xin(Na))] + 435
    - **MELD-Na Score:** is a scoring system (INR, serum bilirubin, creatinine and sodium) created in 2008, based on the original MELD score, but with the addition of serum sodium.(24) The MELD-Na, largely used for prioritisation in the United States, is a better predictor of mortality than the MELD score.
        - MELD-Na = MELD Score - Na - 0.025 x MELD x (140-Na) + 140

      Patients on oral anti-coagulants (including warfarin and the new oral anti-coagulants; predicted to be <2%) will be excluded from the analysis, as their UKELD/MELD-Na will be artificially high. In addition to the pre-LT time-points listed above, UKELD and MELD-Na will be completed on the day admission for LT (immediately pre-LT) and prior to discharge (within 48 hours) from hospital post-LT.
    - **unscheduled hospital admissions:** The frequency and duration (days) of non-elective hospital admissions will be recorded between study baseline (visit 1, week 0) and LT or the 48 week visit. The reason for admission will be categorised based on the serious adverse event (SAE) recording.
    - **mortality:** The date and cause (based on death certificate) of death pre-LT will be recorded.
- **Post-LT morbidity and mortality:** The post-LT morbidity will be assessed by length of ICU stay (hours), length of hospital stay (days; immediately post-LT), and frequency and duration of

hospital re-admission (LT to 24 week visit 9). Of note, the post-LT complications will be captured by the CCI as described above and in addition re-listing for transplant will be recorded (date, reason).

  - **ICU length of stay (hours):** This will be calculated by a member of the trials team (i.e. research nurse; research fellow) using the electronic patient records of the date/time (24hr) of admission to ICU immediately after LT to the date/time (24hr) of discharge from ICU (either through death or transfer to post-LT ward). Re-admission to ICU on the same hospital admission for LT will be included recorded and added to the total ICU length of stay (hours).
  - **length of Hospital stay (LOS; days):** This will be calculated using the date of LT and the date of discharge from hospital to home (either through death or discharge home). In the rare event that a patient is transferred to their local non-LT hospital or an inpatient rehabilitation unit, these bed days will be included in the hospital LOS.
  - **unscheduled Hospital re-admission (frequency, days):** The frequency and duration (days) of non-elective hospital admissions will be recorded between LT and visit 9 (24 weeks). The reason for admission will be categorised based on the serious adverse event (SAE) recording.
  - **mortality (30 day, 90 day, 180 day and 1 year):** The date and cause of death will be documented using the death certificate for reference.

- **Habitual (daily) physical activity times:** Habitual (daily) levels of physical activity engagement (light, moderate and vigorous intensity) which may occur during the course of the trial will be measured using a 'blinded' wrist worn accelerometer (Actigraph GT9X). These will be worn for 14 days (24 hours/day):
  - Baseline (visit 1, week 0) to 14 days
  - 14 days before visit 2 (6 weeks), visit 3 (12 weeks) and visit 4 (24 weeks) pre-LT
  - 14 days before visit 7 (6 weeks post-LT), visit 8 (12 weeks post-LT) and visit 9 (24 weeks post-LT, end of intervention)

  Data captured during the 14 day periods will be analysed to quantify daily time (min/day) spent in; (1) light physical activity (1.6 – 2.9 metabolic equivalents (METS), (2) moderate physical activity (≥ 3 – 5.9 METS), (3) vigorous physical activity (≥6 METS), and sedentary time (≤1.5 METS).
- **"Dose" of exercise completed (measure of the frequency, intensity and duration of exercise):** In addition to the 'blinded' wrist worn accelerometer (Actigraph GT9X) described above, all participants will be asked to wear a heart rate monitor (worn around the chest) during all exercise undertaken as part of either the HBEP (intervention) or the patient exercise advice leaflet (control). This will take place in the same 14 day time periods described above. Data from these devices will be combined (using Actigraph software [Actilife]) to provide an objective measure of the frequency, intensity and duration of structured exercise undertaken in the intervention group and general exercise undertaken in the control group (i.e., the "dose" of exercise undertaken).

- **Adherence to HBEP (intervention arm only)**: As discussed in **section 7.7**, adherence to the HBEP will be measured using the 'blinded' wrist-worn accelerometer and chest worn heart rate monitor worn in the 14 day periods (described above). In addition, the participant will fill a self-reported exercise diary throughout the trial, which will be reviewed by physiotherapist at each face-to-face trial visit until visit 9 (24 weeks post-LT, end of intervention). Using the diary, the physiotherapist will document how many structured HBEP sessions the participant has completed per week (maximum 5 per week).
- **[3]**Perceptions of the health care climate (how need supportive/empowering the physiotherapist is):** This will be measured using the Health Care Climate Questionnaire (HCCQ).(407) The HCCQ comprises 15 items/statements which represent the patient's perceptions of the degree to which they feel their interactions with their physiotherapist (health care climate) empower them to engage in exercise (e.g. "I feel that my physiotherapist has provided me choices and options"). Patients are asked to respond to each item, indicating the extent to which they agree with each statement, on a Likert scale ranging from 1 (strongly disagree) to 7 (strongly agree). For each participant, an average of the 15 items will be calculated for use in analysis. Note: Item 13 is negatively coded, and the score provided will be subtracted from a score of 8 to compute the participants response to this item.
- **[3]**Basic psychological need satisfaction (i.e. feelings of autonomy, relatedness, competence):** This will be measured using the Basic Psychological Need Satisfaction in Exercise Scale (PNES): The PNES will be used to examine participants basic psychological need satisfaction, in relation to their exercise engagement.(408) The PNES comprises 18 items, capturing the three basic psychological needs of autonomy (6 items, e.g. "I feel free to exercise in my own way"), competence (6 items, e.g. "feel that I am able to complete exercises that are personally challenging") and relatedness (6 items, e.g. I feel connected to the people who I interact with while I exercise"). Participants are asked to respond to each item, indicating the degree with which they agree with each statement, on a Likert scale from 1 (false) to 6 (true). Average scores for each of the three subscale will be computed (e.g. 6 items for autonomy are added and divided by 6 to give the average for autonomy need support), to determine participants degree of autonomy, competence and relatedness need support. These individual variables will be used in analysis. In addition, an overall average will also be calculated using responses to all 18 items, to provide and overall PNES score for use in analysis.
- **[3]**Self-determined motivation to exercise:** This will be measured using the Behavioural Regulation in Exercise Questionaire-2 (BREQ-2).(409) The BREQ-2 will measure participant's degree of self-determined motivation to engage in exercise, by assessing their external, introjected, identified and intrinsic regulations, as well as motivation. Following the stem, "I take part in exercise" participants will be asked to respond to 19 items assessing intrinsic regulation (4 items; e.g., "because I enjoy doing this"), identified regulation (4 items; e.g., "because I value the benefits of doing this"), introjected regulation (3 items; e.g., "because I feel guilty when I am not doing this"), external regulation (4 items; e.g., "because my friends and family say I should") and motivation (4 items; e.g., "but I think doing this is a waste of time"). Participants were asked to rate their agreement with each statement on a 5-point Likert scale from 0 (not true for me) to 4 (very true for me). For this study, average scores for each subscale will be computed, and used to produce composite scores for autonomous

motivation (identified regulation + intrinsic regulation) and controlled motivation (external regulation + introjected regulation).

- [3]**Theory-based motivation support programme will be assessed** with (i) HCCQ, (ii) Basic PNES and (iii) BREQ-2 Questionnaires, as discussed above. To test the theoretically-expected psychological mechanisms underlying behaviour change, a theoretical process model of behaviour change will be tested.

[3] ** denotes where analysis will be conducted outside of the BCTU SAP by Prof Joan Duda and Dr Sally Fenton.

Specifically, we will examine whether the intervention (i.e. perceived support for patient autonomy, competence, relatedness by the physiotherapist) predicts change in the targeted psychological determinants (i.e. psychological need support, motivation for exercise), and subsequently, the targeted behaviour including physical activity and QoL. By testing the process model grounded in SDT in this way, we can begin to understand how the experiment intervention has worked. For example: which SDT constructs/determinants did the intervention successfully target/change, in order to encourage behavioural change. This will enable the home-based exercise with motivation support intervention to be subsequently refined and optimised. We will conduct this evaluation using structural equation modelling and path analysis, as previously described.(410, 411)

7.11. Mechanistic 'muscle' sub-study outcomes (n=100):

In addition to the primary/secondary outcome measure (listed above), the mechanistic 'muscle' sub-study outcomes measures will be completed at the pre-LT baseline visit 1 (0 weeks; within 3 days of visit 1), pre-LT visit 2 (6 weeks; +/- 3 days) and post-LT visit 9 (24 weeks post-LT; +/- 7 days). The outcome measures include:

1. **6 minute walk test (6MWT):** The 6MWT is a self-paced field walking test conducted under controlled conditions and is a reliable and valid measure of exercise tolerance in various patient populations.(150) The test is inexpensive and simple to administer. It requires a 30 metre level indoor walking course and the course layout and degree of patient encouragement will be standardised, as they significantly affect the distance walked.(412) The learning effect (i.e. patient becomes more familiar with the test) will be reduced by performing two tests and recording the best result at each study time point. The 6 minute walk distance (6MWD) will be recorded in metres.
2. **Cardiopulmonary exercise testing (CPET):** Cardiopulmonary exercise testing (CPET), using a cyclo-ergometer, is the gold standard assessment tool of aerobic exercise capacity. It directly assesses gas exchange, work, heart rate and rhythm, and blood pressure during intense exercise (162). With the exception of safety reports (i.e. new cardiac arrhythmia), the trial management group, physiotherapists and the patients clinicians will be blinded to the CPET outcome measures until the end of the trial – in order to avoid the results altering the patients clinical course (i.e. as not routine NHS care in QEUHB and RFH). All CPETs will be analysed by an independent assessor at the end of the trial, who will be blinded to the intervention and order of the CPETs. The key CPET outcomes to be measured, include:

- Oxygen consumption at anaerobic threshold (AT; ml/kg/min)
- Peak oxygen consumption (VO_2peak; ml/kg/min)
- Other measures include: ramp rate (W/min; peak power output (W); maximum heart rate (bpm); maximum oxygen pulse (ml/beat); reason for test termination (participant symptoms/request; operators request); exercised to volitional fatigue (YES/NO); ventilatory equivalents for carbo dioxide (VE/CO_2); respiratory exchange ratio at peak exercise.

3. **Right Quadricep muscle size, architecture and quality (ultrasound):** A Two-dimensional B-mode ultrasonography Esoate MyLab^{TM} Alpha point of care ultrasound, 4.6cm probe (SL1543, 13-4Mhz scanning frequency)) will be performed by a member of the clinical trials team (research fellow, physiotherapist, or nominated co-investigator on delegation log). The following will be measured: vastus lateralis [VL) muscle thickness, fascicle pennation angle, fascicle length and total quadricep muscle anatomical cross-sectional surface area [ACSA]). All variables will be obtained offline via image J imaging software and will be presented as a mean. For assessment of all quadricep muscles, two extended field of view ultrasound images will be taken at 50% femur length; this will allow for the quantification of quadriceps ACSA. Echogenicity can be determined using a computer-assisted grey-scale analysis offered by ImageJ. (413) Further details in **section 9.0** Procedures.
4. **Specialist biomarkers:** Blood will be centrifuged, processed and then stored at -80^{o}C at the study sites before being transferred to our specialist laboratory for analysis of the following:
    - Common measures of oxidative stress: Total redox status, malonyldialdehyde, Myeloperoxidase, 4-Hydroxynonenal.
    - Serum antioxidant capacity: catalase, glutathione peroxidase and superoxide dismutase.
    - A profile of key myokines: IL-6, IL-10, IL-15, Irisin, leukaemia inhibitory factor, and secreted protein acidic and rich in cysteine (SPARC)
    - Tumour necrosis factor alpha (not a myokine, but an inflammatory marker).

A full standard operating procedure (SOP) will be produced for the 'muscle' sub-study that details the methodology of all the measures outlined above. Data from this sub-study will be combine with data collected from the main study in order to fully evaluate the effect of the exercise intervention on muscle physiology.

8. TRIAL PROCEDURES

8.12. Study Timelines (estimates)

The total trial length is 54 months and comprises 6 months for trial set-up and contract execution, 24 months for recruitment (estimated 12 patients/month), maximum 18 months intervention (note: minimum = 1 week pre-LT + 6-months post-LT), and 6 months follow-up, primary analysis and reporting.

Prior to the study opening to recruitment, the following will take place:

- 2-3 day physiotherapist training days (home-based exercises; 'Empowering the Physio')
- Application and approval by REC, Sponsor (UoB), local confirmation of capacity and capability (QEUHB, RFH)

8.13. Pre-consent screening at QEUHB and RFH

Pre-consent screening will take place by the transplant coordinators, hepatologists (anaesthetists) and AHPs directly involved in the patients LT waiting list care (**Section 6.1.**). Review of the NHSBT 'active' adult liver transplant waiting list will be undertaken and all potentially eligible patients will either by given the PIS:

- In person at either their liver transplant assessment or in their dedicated liver transplant waiting list clinic/specialist liver clinic (i.e. PSC, HCC)

  OR

- Via post, especially in light of the emergence of telephone/virtual video clinics (as a result of the COVID pandemic)

If a patient expresses an interest to participate in the trial (either via phone, email or in person) they will be invited to attend visit 1 (baseline) of the trial. The study (*where possible*) team will schedule visit 1 (baseline) to be on the same day as their next routine waiting list clinic appointment, during which the patient +/- relative ('personal consultee') will be able to ask questions about the trial before providing consent.

8.14. Phase 1 Pre-LT trial visits schedule

Phase 1 Pre-LT trials will consist of a baseline visit 1 (week 0), visit 2 (6 weeks +/- 3 days), visit 3 (12 weeks +/- 7 days), visit 4 (24 weeks +/- 7 days), visit 5 (36 weeks +/- 7 days) and visit 6 (48 weeks +/- 14 days). These are summarised in **Table 7.**

8.14.16. Pre-LT baseline Visit 1 (week 0) - maximum duration = 3-6 hours

At the beginning of the trial visit 1, **written-consent** will be obtained (by CI/PI or nominated clinical co-investigators on the delegation log) and the **eligibility criteria checklist** will be completed by the research nurse or another nominated member of the clinical trials team (on delegation log):

- NO trial specific examinations, investigations or treatments, that do not involve part of the patient's routine standard healthcare, will be performed prior to obtaining written consent of the patient.
- A member of the site research team (e.g. CI/PI or designated co-investigator as documented on the signature and delegation log) will discuss with patient, all of the relevant study information including aims, methods, risk and benefits of the trial, prior to obtaining consent.

- At this stage the patient will also be asked nominate potential **'personal consultee(s)'** in the event that they lack capacity at any stage of the trial. A contact list of the participant's nominated personal consultee(s) will be securely stored at the trial site.
- Eligibility and confirmation of consent (including version of ICF, date, time) will be documented in the participants clinical noting.

After eligibility has been confirmed and informed consent given, the patient will be randomised (1:1) to either the study intervention (Group 1) or control (Group 2) (**section 6.3**) and provided with a unique **Study Identification Number** (ID). The study ID number will be used on all future trial documentation, alongside the patients initials, date and visit number.

The following **data collection** will take place at visit 1:

- **Patient demographics:** Patient's name, Age, DOB, gender, ethnicity; post code, local hospital (non-LT centre).
- **Hospital trial site** (QEUHB, RFH)
- **Full course of COVID vaccination**; Previous positive PCR test for COVID
- **Current medical history and clinical examination**, including: hepatic encephalopathy, ascites (defined as - mild (imaging only), moderate (on examination), severe (tense, requiring large volume paracentesis [LVP]), diuretic-resistant or diuretic-intolerant ascites, LVP (frequency), peripheral oedema, jaundice, haematemesis/melaena (< 4 weeks), pruritis, fever, right upper quadrant abdominal pain, fatigue, tiredness.
- **Past medical history:**
    - *primary chronic liver disease*
        - type (NAFLD/MAFLD, ArLD, Cryptogenic, PSC, AIH, PBC, A1AT, HBV, HCV, other)
        - HCC
            - largest Size
            - number of active lesions
            - previous treatment (TACE, RFA, SABR)
        - portal Hypertension
        - ascites
        - hepatic encephalopathy
        - spontaneous bacterial peritonitis (SBP)
        - hepatopulmonary syndrome
        - main portal vein thrombosis
    - *listing for LT (at the time)*
        - date of listing with NHSBT
        - indication (UKELD >/= 49, HCC, Variant Syndrome i.e. recurrent cholangitis, refractory ascites)
        - UKELD score, MELD-NA
        - blood Group
        - listing graft choice (DBD, DCD, or both)

- **Current/recent illnesses (<6 weeks)**, specifically: SBP, variceal haemorrhage (melaena, haematemesis), hepatorenal syndrome/Acute Kidney Injury (AKI), sepsis, severe hepatic encephalopathy (admission to hospital), chest pain, shortness of breath, palpitations, dizziness/collapse, significant fall
- **Significant co-morbidities**: diabetes (Type 2 or Type 1; duration; insulin; retinopathy; neuropathy; proteinuria), hypertension; atrial fibrillation/atrial flutter, ischaemic heart disease (IHD), cerebrovascular disease (CVD), pulmonary disease (asthma, COPD, interstitial lung disease); musculoskeletal disease, mental health illness.
- **Drug History (key), including:** diuretics, beta-blockers, anti-encephalopathy medication, SBP prophylaxis, anti-depressants/anti-anxiolytic, sleeping adjuncts, analgesia, anti-diabetic drugs, long-term antibiotics, anti-pruritus, anti-coagulants.
- **Nutritional supplements** (prescribed amount):
    - oral
    - enteral
    - additional supplements
        - Creon/nutrizyme/pancrex
        - vitamin D
- **Social history**:
    - smoking status
    - alcohol intake
    - employment status
    - living situation

The following **assessments** (nurses checklists, CRFs) will take place at visit 1:

- **Full clinical examination** (including general, cardiovascular, respiratory, abdominal, neurological)
- **Clinical Observations,** including:
    - blood pressure (mm/Hg) – patient sitting 2 minutes prior.
    - resting pulse (beats/min)
    - oxygen Saturations on room air (%) – sitting
    - temperature (^{o}C)
- **Nutrition/Physical/functional status**:
    - wet weight (kg)
    - height (cm)
    - wet BMI (kg/m2)
    - estimated dry weight (kg) [*using the 5/10/15% reduction rule for mild/moderate/severe ascites; 5% for peripheral oedema*]
    - estimated dry BMI (kg/m2)
    - handgrip strength (HGS; dominant hand)
    - MAMC (cm)
- **LFI** (range 1.5 to 7.5; record in 2dp)
    - HGS: mean = ……. Kg (as above – autofill from above)
    - time to do 5 chair stands = …… seconds (NB if can't do record as '0')

- balance (feet); total = …… (maximum 30 secs)
    - side = XX/10 secs
    - semi-tandem = XX/10 secs
    - tandem = XX/10 secs

The following **questionnaires** will take place at visit 1:

- DASI:
    - DASI Points =……. (range 0-58.2)
    - VO_2 peak (ml/kg) = 0.43 x DASI points + 9.6 = …….ml/kg (range 9.6-34.6 automated)
    - Metabolic equivalents (METs) = VO2 peak/3.5 = ……(range 2.74-9.89)
- SF-36v2 Health Survey (Quality of Life Questionnaire) [ ]:
    - Total score = [range 0-100]
    - PCS= [range 0-100; PRIMAY END-POINT MEASURE]
    - MCS= [range 0-100]
    - these will be batch calculated using the Quality Metric software as described above.
- HCCQ
- Basic PNES
- BREQ-2

The following **investigations** will take place at visit 1:

- **Urine ACR** (*white top urine bottle to biochemistry*) – *visit 1 only*
- **12-lead ECG** (if not within previous 6 weeks) – *visit 1 only*
    - rate (beats per minute)
    - rhythm
- **Blood samples** (*non-fasted*):
    - haematology: FBC, reticulocytes, Prothrombin Time (PT), INR
    - biochemistry: Ferritin, Trans Sats (%), B12, Folate, Vitamin D, Calcium (Adjusted Ca)
    - biochemistry: Urea & Electrolytes (including magnesium, phosphate), liver function tests (LFTs) including AST, GGT
    - biochemistry: HbA1c, TFTs (TSH, T4), lipid profile (Total cholesterol, HDL, triglyceride)
    - biochemistry: AFP, CRP, Alcohol (*if listed for ArLD*)
    - biochemistry: Ammonia (*on ice to laboratory within 15 minutes of collection*)
- The following with be calculated (study automated by eCRF):
    - UKELD* *if on oral anticoagulant – result will be void.
    - MELD-Na*
    - Childs-Pugh score* (5-15)

The following **additional <u>mechanistic 'muscle' sub-study'</u> investigations** will take place at visit 1 (*if consented for sub-study*) in the ***following order***:

1. **Specialist biomarkers:** Blood (*non-fasted*) will be collected in 2 x purple top tubes (3ml each) and 3 x red top tubes (5ml each). Samples will be initially stored at site until they are they are sent to a laboratory at the University of Birmingham where they will be processed, centrifuged, stored at 80^{o}C and then batch analysed. All plasma and serum samples will be labelled with

unique study ID, site, date, visit number and patient initials. Batch transfer and analysis will take place for measures of oxidative stress, anti-oxidant capacity, myokines and inflammatory markers as listed in **section 8.0**.

2. **Right Quadricep Muscle size, architecture and quality (Ultrasound):** A Two-dimensional B-mode ultrasonography Esoate MyLab^{TM} Alpha point of care ultrasound, 4.6cm probe (SL1543, 13-4Mhz scanning frequency)) will be performed by a member of the clinical trials team (research fellow, physiotherapist, or nominated co-investigator on delegation log) prior to any significant functional tests (i.e. 6MWT, CPET). The following will be measured: VL muscle thickness, fascicle pennation angle, fascicle length and total quadricep muscle ACSA. For assessment of all quadricep muscles, two extended field of view ultrasound images will be taken at 50% femur length; this will allow for the quantification of quadriceps ACSA.
3. **6MWT (supervised by the study AHPs or CRF/PIs):** 30 metre level indoor walking course and the course layout and degree of patient encouragement will be standardised. 'The learning effect' (i.e. patient becomes more familiar with the test) will be reduced by performing two tests and recording the best result. **6-minute walk distance (6MWD)** will be recorded in metres (range 50 to 2000 metres). In addition, participants will wear a pulse oximeter throughout the 6MWT and the starting SaO_2 on room air (%) and lowest SaO_2 on room air (%) will be recorded. There will be a minimum of 15 minutes rest time prior to the CPET.
4. **Cardiopulmonary exercise testing (CPET):** CPET, using a cyclo-ergometer, will be performed to directly assess gas exchange, work, heart rate and rhythm, and blood pressure during intense exercise. With the exception of safety reports (i.e. new cardiac arrhythmia), the trial management group, physiotherapists and the patients clinicians will be blinded to the CPET outcome measures until the end of the trial. A SOP for CPET will be used at both trial sites to ensure standardised methodology for collaborating equipment, preparing the patient (mask fitting, seat height), software set-up, safety rules (i.e. contraindications, stopping rules), cycling/resting protocol and data storage. Participants will be encouraged to maintain a cycling speed of 60-65 rpm for as long as they can, whereby it will start easy and become more difficult as the resistance increases. The key CPET outcomes to be measured are summarised in **section 8.0** and will be determined by an independent, trained 'blinded' physiologist at a later date using the methods described in the POETTS guidelines.(414)

<u>Study Intervention:</u>

After completion of the baseline assessments, participants will have a face-to-face consultation with the study physiotherapist:

- **Intervention Group 1 (see section 7.3):** A 60-minute consultation with the physiotherapist, during which the entry level of difficulty for the HBEP (aerobic and resistance) will be determined. The participant will be provided with a personalised written HBEP. The physiotherapist will record and save personalised written HBEP in the site file and patients clinical noting. The participants will then attend a physiotherapist delivered training session (group session; maximum 4 participants/day), which will consist of: a) patient education (1 hour), b) exercise familiarisation (1 hour) and c) issuing of devices and written information (1 hour).

- **Control Group 2 (see section 7.5)**: A 20-minute consultation with the physiotherapist, during which they will receive verbal and written (patient leaflet) advice on the generic benefits of exercise pre-LT.

Exercise Monitoring (**section 7.7**):

The physiotherapist will issue participants in both the intervention and control groups with:

- **A participant 'exercise' diary (paper booklet)**: for them to self-record all structured exercise undertaken during the trial.
- **A 'blinded' wrist worn accelerometer (Actigraph GT9X)**: for them to wear for the next 14 days (Days 1-14; 24 hours/day) days at baseline. Participants will also be asked to wear the accelerometer for 14 days prior to visit 2 (week 6) and bring it to visit 2. Participants will be asked to contact the research team should they have any queries regarding accelerometer wear.
- **A chest strap heart rate monitor (Actigraph):** for them to wear during any structured exercise they undertake during the trial period (i.e. during either aerobic or resistance exercises). Data from the heart rate monitor will be downloaded and stored at visit 2.

8.14.17. Pre-LT Visit 2 (week 6 +/- 3 days) - maximum duration = 30-60 mins*

Visit 2 will take place alongside the participants routine LT waiting list clinic (*where possible*). At visit 2

the following will take place:

- **Record 'new' clinical events since last visit: Current/recent illnesses (<6 weeks)**, specifically: hepatic encephalopathy (I-IV), ascites (moderate to severe), number of LVP, peripheral oedema, variceal haemorrhage (requiring endoscopy), hepatorenal syndrome/AKI, sepsis/fever, significant fall.
- **Record Serious Adverse Events** (SAEs)**:** non-elective hospitalisation (Days in hospitals; if '0' = no hospitalisation); requirement for organ support/intensive care, hepatorenal syndrome/AKI, severe hepatic encephalopathy, variceal haemorrhage, serious fall/musculoskeletal injury, cardiac or cerebrovascular event, sepsis/infection requiring admission (esp. SBP, pneumonia).
- **Record 'new' medications:** diuretics, beta-blockers, anti-hepatic encephalopathy medications, SBP prophylaxis, antibiotics (not SBP prophylaxis), anti-depressants/anti-anxiolytics, sleeping adjuncts.
- **Record Nutritional supplements (prescribed amount)** – as per visit 1
- **Full examination, clinical observations and nutritional/physical/functional (including LFI)** – as per visit 1
- **Questionnaires** (DASI, SF-36v2, HCCQ, Basic PNES, BREQ-2) – as per visit 1
- **Blood tests** (including UKELD, MELD-Na, Childs-Pugh) – as per visit 1
- **Mechanistic 'muscle' sub-study'_investigations** (if consented for 'sub-study'; biomarkers, muscle ultrasound, 6MWT, CPET) – as per visit 1
- **Study Intervention:**

- ***Intervention Group 1***: The study physiotherapist will progress exercises and revise the level/goals of the HBEP after review of the LFI/DASI assessments, participant 'exercise' diary and discussions with the participant themselves will be used to progress exercises and revise goals of their HBEP. As per 'Empowering Physio' principles strategies, the active role of the participant in the decision-making process, regarding progression and goal revision, will continue to support more autonomous reasons for engagement in the HBEP. The updated written HBEP will be saved in the trial site file and recorded in clinical notes.
- ***Control Group 2***: participant will have an opportunity to discuss any concerns regarding physical activity or exercise they have with the physiotherapist (15 minutes). However, the physiotherapist will only provide information in line with established generic physical activity and exercise guidelines on the patient exercise leaflet.

- **Exercise monitoring**: The participant will return the wrist-worn accelerometer, chest strap heart rate monitor and the participant 'exercise' diary. A member of research team will then download the data from the devices, and securely store it at the trial site.
- **Preparation for next trial visit (3):**
  - at the end of the trial visit, the participant will be given a time/date for their next appointment.
  - prior to the next trial visit the research team will post the accelerometer and heart rate monitor (during exercise only) out to the participant for them to wear for the 14 days prior to the next trial visit (i.e. visit 3; 12 weeks).

Table 7: ExaLT trial visit schedule

*48-week cut-off: study intervention will stop if participant hasn't had their LT by 48weeks

	BASELINE										End-of-Interventio	FOLLOW-UP
	Pre-Liver Transplant (variable time-line)						TRANSPLANT	Post-Liver Transplant (fixed time-line)				
Study visits	VISIT 1	VISIT 2	VISIT 3	VISIT 4	VISIT 5	VISIT 6			VISIT 7	VISIT 8	VISIT 9	VISIT 10
Time points	0 weeks	6 weeks	12 weeks	24 weeks	36 weeks	48 weeks	*48 week cut-off	Inpatient stay	6-weeks post	12 weeks post	24 weeks post	48 weeks post
		(+/- 3 days)	(+/- 7 days)	(+/- 7 days)	(+/- 7 days)	(+/- 14 days)		day 10 +/- 3 days	(+/- 7 days)	(+/- 7 days)	(+/- 14 days)	(+/- 14 days)
Consent	X											
Randomisation (Intervention vs. control))	X											
Clinical examination and review (routine clinic)	X	X	X	X	X	X	X	X	X	X	X	X
Standard bloods pre-LT (FBC, U+E, LFTs, AST, GGT, INR, CRP, nutrition, ammonia)	X	X	X	X	X	X	X					
Standard bloods post-LT (FBC, U+E, LFTs, AST, GGT, INR, CRP, nutrition, tacrolimus)								X	X	X	X	X
Standard clinic observations (BP, dry BMI, weight, hand grip strength, MAMC)	X	X	X	X	X	X	X	X	X	X	X	X
Frailty/Functionality assessment (LFI, DASI)	X	X	X	X	X	X	X	X	X	X	X	X
Primary outcome SF-36v2 questionnaire	X	X	X	X	X	X		X	X	X	X	X
Behavioural/psychological questionnaires (HCCQ, PNES, BREQ-2)	X	X	X	X	X	X		X	X	X	X	X
Serious adverse events (complications/morbidity)	X	X	X	X	X	X	X	X	X	X	X	X
Liver Transplant data (date, donor details, organ support, ICU stay)							X					
Exercise Adherence: review participant 'exercise' diary		X	X	X	X	X	X		X	X	X	X
Exercise Adherence: Accelerometer/Heart rate monitor	week 0-2	4-6	10-12	22-24					week 4-6	10-12	22-24	
Mechanistic 'muscle' sub-study (n=100, *optional*):												
CPET, muscle USS, 6MWT, specialist biomarkers	X	X									X	
Interventions:												
Intervention: home-based exercise and theory-based motivation support programme	X	X	X	X	X	X		X	X	X	X	
Intervention: Telecall (15-30 minutes)		week 2,4	8, 10	16,20					4	8,10		
Control: standard of care patient 'exercise' advice leaflet	X							X				

8.14.18. Pre-LT Visits 3 (week 12 +/-7 days), 4 (week 24 +/-7 days), 5 (week 36 +/- 7 days) – max. 60min

Visits 3-5 will take place alongside the participants routine LT waiting list clinic (*where possible*). All procedures, data collection, and study intervention will be the same as visit 2, without the mechanistic 'muscle' sub-study investigations.
Similarly to visit 2, at the end of the trial visit, the participant will be given a time/date for their next appointment and the participant 'exercise' diary. Prior to the trial visits 3 and 4 only the research team will post the accelerometer and heart rate monitor out to the participant for them to wear for the 14 days prior to the next trial visit.

8.14.19. Pre-LT Visit 6 (week 48 +/- 14 days) – max. 60 min

Visits 6 will take place alongside the participants routine LT waiting list clinic (*where possible*). At visit 6 (week 48 +/- 14 days), the following will be undertaken:

- **Record 'new' clinical events since last visit: Current/recent illnesses (<6 weeks)** – as per visit 2
- **Record Serious Adverse Events** (SAEs only) – as per visit 2
- **Record 'new' medications** – as per visit 2
- **Record Nutritional supplements (prescribed amount)** – as per visit 1
- **Full examination, clinical observations and nutritional/physical/functional (including LFI)** – as per visit 1
- **Questionnaires** (DASI, SF-36v2, HCCQ, Basic PNES, BREQ-2) – as per visit 1
- **Blood tests** (including UKELD, MELD-Na, Childs-Pugh) – as per visit 1
- The participant will return the **participant 'exercise' diary**.

However, if the participant has not had a LT by trial visit 6 (48 weeks +/- 14 days within randomisation) they will be **withdrawn from the study intention**. The rate of study intervention withdrawal at this stage has been powered for in the sample size calculation (i.e. approximately 30-35% of the randomised cohort). On this visit, the participant will have the option of asking questions and discussing their current HBEP with the study physiotherapist (maximum 20 minutes). The patient will also have the option to consent to post-LT data collection (inc. 24 week post-LT SF-36v2) if they undergo LT whilst the ExaLT trial is still ongoing. There will, however, be no protocolised study intervention after visit 6 or scheduled post-LT trial visits. The research team will ensure that the patient has a time/date for their next LT waiting list clinic appointment.

8.14.20. Pre-LT Telecalls at weeks 2, 4, 8, 10, 16 and 20

Telecalls will be performed by the study physiotherapist at weeks 2, 4, 8, 10, 16 and 20 (**Table 7**). The purpose of these calls (duration 15-30 minutes) will be to: identify any adverse events or areas of concern; gain feedback from the participant regarding the HBEP; and provide motivational support for engagement with the HBEP through the implementation of *Empowering Physio* strategies (**Table 2**).

8.15. Day LT (unpredictable timing) – trial data collection

The following (below) investigations and data collection will take place on the admission for LT (i.e. prior to the LT). If the participant is admitted overnight, the data (where possible) will be collected in the morning prior to the LT:

- **Record 'new' clinical events since last visit: Current/recent illnesses (<6 weeks)**, specifically: hepatic encephalopathy (I-IV), ascites (moderate to severe), number of LVP, peripheral oedema, variceal haemorrhage (requiring endoscopy), hepatorenal syndrome/AKI, sepsis/fever, significant fall.
- **Record SAEs**: non-elective Hospitalisation (Days in hospitals; if '0' = no hospitalisation); requirement for organ support/intensive care [yes/no]; hepatorenal syndrome/AKI, severe hepatic encephalopathy, variceal haemorrhage, serious fall/musculoskeletal injury, cardiac or cerebrovascular event, sepsis/infection requiring admission (esp. SBP, pneumonia).
- **Record 'new' medications:** diuretics, beta-blocker, anti-hepatic encephalopathy medications, SBP prophylaxis, antibiotics (not SBP prophylaxis], anti-depressants/anti-anxiolytics, sleeping adjuncts.
- **Record Nutritional supplements (prescribed amount)** – as per visit 1
- **Full examination, clinical observations and nutritional/physical/functional (including LFI)** – as per visit 1
- **DASI Questionnaire (***if possible***)** – NB no other questionnaires will be performed immediately prior to LT, as high risk of inaccuracy due to the emotional stress associated with waiting for high risk surgery (LT).
- **Blood tests** (including UKELD, MELD-Na, Childs-Pugh) – as per visit 1

8.16. Phase 2 Post-LT trial schedule (inpatient stay, visits 7-9)

8.16.21. LT data capture

The following data will be obtained by a member of the research team from the LT operation note, anaesthetic chart, ICU charts, clinical noting and the NHSBT database (*if required*) (**Table 7**):

- Date of LT
- Donor and operation details:
    - type of donor
    - type of graft
    - ABO match
    - donor age
    - cytomegalovirus (CMV) donor status
    - graft steatosis
    - normothermic machine perfusion
        - duration on the machine
    - cold ischaemic time
    - operation time

    - renal replacement therapy intra-operative; continuous veno-venous hemofiltration (CVVH)
    - recipient cardiac arrest in theatre
    - blood products in theatre
- ICU stay (time of admission to ICU to time of discharge to post-LT ward):
    - length of ICU stay (hours)* includes re-admission to ICU on the index post-LT hospital admission
    - duration of invasive ventilation (hours) = time of admission to ICU to time of extubation.
    - post-LT lactate (1st lactate on return to ICU after LT)
    - duration of inotropes
    - CVVH (including duration)
- Immunosuppression regimen (including renal-sparing regimen if required)

8.16.22. Phase 2 Post-LT inpatient surgical ward (post-ICU step-down)

The following (below) investigations and data collection will take place post-LT on the inpatient surgical ward (post ICU step-down):

- **Record LT Surgical complications/SAEs (frequency, CTCAE grade)**, **including**: biliary stricture/anastomosis, bile leak, wound dehiscence, abdominal collection (requiring drainage/intravenous antibiotics), haematoma/bleeding (requiring surgical/radiological intervention), anaemia requiring blood transfusion, AKI (requiring renal replacement therapy), Ileus/bowel obstruction, re-laparotomy, portal vein thrombosis, hepatic artery (or conduit) thrombosis, bacteraemia requiring intravenous antibiotics, hyperglycaemia requiring insulin infusion, infection (pneumonia; cholangitis; urinary tract; wound; peritonitis; central nervous system; CMV), acute rejection (biopsy proven)
- **Record** any of the following:
    - re-admission to ICU
    - death (including cause)
    - re-transplantation
- **Record new medications** (specific; prior to discharge): anti-depressants/anti-anxiolytics, prescribed sleeping adjuncts, analgesics.
- **Blood (non-fasted) tests on post-LT day 1,3 and 7 (+/-1 day):**
    - FBC (full blood count; inc. neutrophils, lymphocytes, eosinophils, prothrombin time (PT) and INR
    - urea and electrolytes (inc. magnesium and phosphate). Document if on renal replacement support.
    - LFTs including AST
    - peak ALT (IU/L)
    - C-reactive protein (CRP)
    - tacrolimus trough level (if applicable)
- **Day prior to discharge or day 10 post-LT +/- 3 days record nutrition/Physical/functional status:**

- wet weight (kg)
- height (cm)
- wet BMI (kg/m2)
- estimated dry weight (kg) [*using the 5/10/15% reduction rule for mild/moderate/severe ascites; 5% for peripheral oedema*]
- estimated dry BMI (kg/m2)
- nutrition management:
  - route (inc. protein intake)
  - oral supplements (inc. protein intake)
  - normal diet
- Handgrip strength (HGS; dominant hand)
  - 1st ……..2nd……..3rd……Mean =…….Kg
- MAMC (cm; *if possible*)
- **LFI** (range 1.5 to 7.5; record in 2dp) by member of the research team (nurse, physiotherapist, CRF)
- **DASI questionnaire**
- **SF-36v2 Health Survey (Quality of Life Questionnaire)**

- **Record the length (days) of index hospital stay post-LT** (inc. transfer to other hospitals)
- **Record discharge destination** (home, rehabilitation unit, other hospital, care facility)

Study Intervention post-LT:

The trial physiotherapists will review all participants on the post-LT ward, within 48 hours of discharge from ICU and will start a supervised (basic) exercise programme, based upon the participant's current level of physical frailty/function, in keeping with post-surgical care (**see section 7.4.1**). If there are concerns by the physiotherapist about the participant's safety to exercise (i.e. walk, chair stand etc), the participant's consultant (i.e. Transplant surgeon, hepatologist) and clinical team (i.e. nurse) will be consulted – as per routine NHS care.

The post-LT baseline assessments (LFI and DASI) undertaken prior to discharge (or day 10 post-LT +/- 3 days), along with "Empowering Physio" strategies, will be used to prescribe a personalised written HBEP for the participant post-LT (*intervention group 1 only*). The control group will be given the post-LT patient 'exercise' advice leaflet prior to discharge. All participants will also be given a participant 'exercise' diary to self-report exercises undertaken between discharge and visit 7.

Preparation for next trial visit 7 (6-weeks post-LT):

Prior to discharge, the participant will be given a time/date for their next trial visit appointment, which where possible will be on the same day as their routine post-LT clinic. Prior to the next trial visit (visit 7) the research team will post the accelerometer and heart rate monitor (for during exercise only) out to the participant for them to wear for the 14 days prior to the next trial post-LT visit 7.

8.16.23. Phase 2 post-LT visit 7 (6 weeks +/- 7 days) and visit 8 (12 weeks +/- 7 days) – max. 60 min

These visits will take place alongside the participant's routine post-LT clinic (*where possible*). The following investigations and data collection will take place at each visit (**Table 7)**:

- **Record 'new' clinical events** (since discharge/last visit): ascites, peripheral oedema, jaundice, confusion/delirium, fever/night sweats, severe gastrointestinal symptoms, significant fall/injury.
- **Record LT surgical complications/SAEs (frequency, CTCAE grade)**, **including**: biliary stricture/anastomosis, bile leak, wound dehiscence, new onset ascites (requiring LVP, admission), abdominal collection (requiring drainage/intravenous antibiotics), haematoma/bleeding (requiring surgical/radiological intervention), anaemia requiring blood transfusion, AKI (requiring renal replacement therapy), ileus/bowel obstruction, re-laparotomy, portal vein thrombosis, hepatic artery (or conduit) thrombosis, bacteraemia requiring intravenous antibiotics, hyperglycaemia requiring insulin infusion, infection (pneumonia; cholangitis; urinary tract; wound; peritonitis; CNS; CMV), acute rejection (biopsy proven*), other (e.g. seizure, cardiac/CVA):
- **Record** any of the following:
    - re-admission to ICU
    - death (inc. cause)
    - re-transplantation
- **Record new medications** (specific; prior to discharge): anti-depressants/anti-anxiolytics, prescribed sleeping adjuncts, analgesics.
- **Immunosuppression regimen** (including renal-sparing regimen if required)

The following **assessments/investigations** (nurses checklists, CRFs) will take place at visits 7 and 8:

- **Full clinical examination** (including general, cardiovascular, respiratory, abdominal, neurological)
- **Clinical Observations** – as per visit 1
- **Nutrition/Physical/functional status** – as per visit 1
- **LFI** – as per visit 1
- **Questionnaires** (DASI, SF-36v2, HCCQ, Basic PNES, BREQ-2) – as per visit 1
- **Blood tests (non-fasted):**
    - FBC (inc. neutrophils; lymphocytes; eosinophils), Prothrombin Time (PT), INR
    - ferritin, trans sats (%), B12, folate, vitamin D, calcium (adjusted)
    - urea and electrolytes (inc. magnesium, phosphate)
    - LFTs including AST
    - CRP
    - HbA1c
    - tacrolimus – trough level (if applicable)

Study Intervention:

After completion of the assessments/investigations, participants will have a face-to-face consultation with the study physiotherapist:

- **Intervention Group 1 (see section 7):** A 30-60-minute consultation with the physiotherapist. As per phase one, the physiotherapist will use the assessments (LFI, DASI) along with "Empowering Physio" techniques and the participant's owned perceived progress to revise their personalised HBEP. The participant will be provided with a personalised written HBEP. The physiotherapist will record and save personalised written HBEP in the site file and patients clinical noting.
- **Control Group 2 (see section 7)**: As per phase 1 of the control arm, the physiotherapist will continue providing the advice highlighted in the patient 'exercise' advice leaflet and advise the participant to continue recording any formal exercise sessions in their participant diary.

Exercise Monitoring (**section 7.7**):
The physiotherapist will issue participants in both the intervention and control groups with:

- **A participant 'exercise' diary (paper booklet)**: for them to self-record all structured exercise undertaken during the trial.
- **An 'blinded' wrist worn accelerometer (Actigraph GT9X)**: for them to wear for the next 14 days before visits 8 and 9. Participants will also be asked to wear the accelerometer for 14 days prior to these visits and bring it to the visit. Participants will be asked to contact the research team should they have any queries regarding accelerometer wear.
- **A chest strap heart rate monitor (Actigraph):** for them to wear during any structured exercise they undertake during the trial period (i.e. during either aerobic or resistance exercises). Data from the heart rate monitor will be downloaded and stored at the face-to-face visits.

Preparation for next trial visit 9 (24-weeks post-LT):
The participant will be given a time/date for their next trial visit appointment, which where possible will be on the same day as their routine post-LT clinic.

8.16.24. Post-LT Telecalls at weeks 4, 8 and 16

Telecalls will be performed by the study physiotherapist at weeks 4, 8, and 16 post-LT (**Table 7**). The purpose of these calls (duration 15-30 minutes) will be to: identify any adverse events or areas of concern; gain feedback from the participant regarding the HBEP; and provide motivational support for engagement with the HBEP through the implementation of *Empowering Physio* strategies (**Table 2**).

8.16.25. Post-LT (End of study intervention) Visit 9 (24 weeks +/- 14 days)

The end of intervention visit (visit 9) will take place alongside the participants routine post-LT clinic (*where possible*). The following investigations and data collection will take place at this visit:

- **Record 'new' clinical events** (since discharge/last visit): ascites [mild, moderate or severe], peripheral oedema, jaundice, confusion/delirium, fever/night sweats, severe gastrointestinal symptoms (Vomiting/diarrhoea/nausea/loss of appetite); significant Fall/injury or NONE [ ]
- **Record LT Surgical complications/SAEs (frequency, CTCAE grade)**, **including**: biliary stricture/anastomosis, bile leak, wound dehiscence, new onset ascites (requiring LVP, admission), abdominal collection (requiring drainage/intravenous antibiotics), haematoma/bleeding (requiring surgical/radiological intervention), anaemia requiring blood

transfusion, AKI (requiring renal replacement therapy), Ileus/bowel obstruction, re-laparotomy, portal vein thrombosis, hepatic artery (or conduit) thrombosis, bacteraemia requiring intravenous antibiotics, hyperglycaemia requiring insulin infusion, infection (Pneumonia; Cholangitis; Urinary tract; Wound; Peritonitis; CNS; CMV), other (record), acute rejection (biopsy proven), other (i.e. seizure, cardiac/CVA event).

- **Record** any of the following:
    - re-admission to ICU
    - death (inc. cause)
    - re-transplantation
- **Record new medications** (specific; prior to discharge): anti-depressants/anti-anxiolytics, prescribed sleeping adjuncts, analgesics.
- **Immunosuppression regimen** (including renal-sparing regimen if required)

The following **assessments/investigations** (nurses checklists, CRFs) will take place at visit 9:

- **Full clinical examination** (including general, cardiovascular, respiratory, abdominal, neurological) – key capture = jaundice; ascites; peripheral oedema; walking aids (stick, frame, wheelchair)
- **Clinical Observations** – as per visit 1
- **Nutrition/Physical/functional status** – as per visit 1
- **LFI** – as per visit 1
- **Questionnaires** (DASI, SF-36v2, HCCQ, Basic PNES, BREQ-2) – as per visit 1
- **Blood tests (non-fasted)** – as per visits 8 and 9
- The following **additional <u>mechanistic 'muscle' sub-study'</u> investigations** (nurses checklists, CRFs) will take place at visit 1 (*if consented for sub-study*) in the *following order*:
    - **specialist biomarkers** – as per visits 1 and 2
    - **right Quadricep Muscle size, architecture and quality (Ultrasound)** – as per visits 1 and 2.
    - **6MWT (supervised by the study AHPs or CRF/PIs)** – as per visits 1 and 2.
    - **CPET** – as per visits 1 and 2

<u>End of study Intervention:</u>

After completion of the assessments/investigations, participants will have a face-to-face consultation with the study physiotherapist:

- **Intervention Group 1 (see section 7):** This visit will mark the <u>end of the study intervention</u> delivered by the physiotherapists. No further personalised written HBEP will be provided. The participant will have a face-to-face consultation with the study physiotherapist. "Empowering Physio" techniques will be finalised and the participant will be given advice on the following domains to promote long-term motivation/engagement with exercise after the study intervention:
    - decisional balance patient-centred goal setting
    - supports attempts to change behaviour
    - normalised failed attempts

- problem-solving

- **Control Group 2 (see section 7)**: No further patient 'exercise' advice leaflets will be provided.

Exercise Monitoring (**section 7.7**):
The physiotherapist will collect, store and download (if electronic) the participants:

- **Participant 'exercise' diary (paper booklet)**
- **'Blinded' wrist worn accelerometer (Actigraph GT9X)**
- **Chest strap heart rate monitor (Actigraph)**

Preparation for next trial visit 10 (48-weeks post-LT):
The participant will be given a time/date for their next trial visit appointment, which where possible will be on the same day as their routine post-LT clinic. The participant will be provided with a participant 'exercise' diary and encouraged to continue to record any exercises between visits 9 and 10.

8.17. Post-LT (follow-up) Visit 10 (48 weeks +/- 14 days) – max. 60 mins

The end of study follow-up visit 10 (post-LT +/- 14 days) will take place alongside the participants routine post-LT clinic (*where possible*). The following investigations and data collection will take place at this visit:

- **Record LT Surgical complications/SAEs (frequency, CTCAE grade)**, **including**: biliary stricture/anastomosis, bile leak, wound dehiscence, new onset ascites (requiring LVP, admission), abdominal collection (requiring drainage/intravenous Antibiotics), haematoma/bleeding (requiring surgical/radiological intervention), anaemia requiring blood transfusion, AKI (requiring renal replacement therapy), Ileus/bowel obstruction, re-laparotomy, portal vein thrombosis, hepatic artery (or conduit) thrombosis, bacteraemia requiring intravenous antibiotics, hyperglycaemia requiring insulin infusion, infection (Pneumonia; Cholangitis; Urinary tract; Wound; Peritonitis; CNS; CMV), other (record), acute rejection (biopsy proven*)
    - *mild [ ] moderate [ ] severe [ ]
    - pulsed steroids [ ]
    - T-cell [ ] AMR (+DSA) [ ]

  Other (ie. Seizure, cardiac/cva event; reason for re-admission to hospital):
- **Record** any of the following:
    - re-admission to ICU (YES/NO)
    - death: Date XX/XX/XX; Cause of death Certificate: 1a……. 1b……. 2……
    - re-transplantation: Date XX/XX/XX

The following **assessments/investigations** (nurses checklists, CRFs) will take place at visit 10:

- **Full clinical examination** (including general, cardiovascular, respiratory, abdominal, neurological)
- **Clinical Observations** – as per visit 1
- **Nutrition/Physical/functional status** – as per visit 1

- **LFI** – as per visit 1
- **Questionnaires** (DASI, SF-36v2, HCCQ, Basic PNES, BREQ-2) – as per visit 1
- **Blood tests (non-fasted)** – as per visit 9

At the end of trial visit 10 (end of study), a member of the research team will:

- Collect/store the participant 'exercise' diary.
- Ensure the patient has a date for their next routine NHS post-LT clinic appointment.
- Research team will inform in writing the GP, LT units clinician and the local hospitals hepatologist that the patient has completed the trial.
- The patient will be thanked for their participation and will be informed of any future trial results, conclusions and publications in writing (or via the PPI and patient support groups).

8.18. Withdrawal and changes in levels of participation

Informed consent is defined as the process of learning the key facts about a clinical trial before deciding whether or not to participate. It is a continuous and dynamic process and participants will be asked about their ongoing willingness to continue participation at all visits. Participants will be made aware from the beginning of the trial that they can freely withdraw (cease to participate) from the trial at any time. A participant may wish to cease to participate in a *particular* aspect of the trial (i.e. study intervention, sub-study), but give consent to participate in the remaining trial visits and outcome measure data collection. The date and reason the patient withdraws consent for a *particular* aspect of the trial (state 'reason unknown' if no reason provided) will be clearly documented in the patient's medical notes.

To enable enrolment into the ExaLT trial, all patients must give consent to participate in the pre-LT and post-LT treatment period, follow-up appointments and compliance with investigations required for treatment efficacy and safety monitoring. At any stage between randomisation and the 48-week post-LT visit, a patient may withdraw consent from being a participant in trial, without necessarily giving a reason and without any personal disadvantage. The details of withdrawal will be clearly documented and communicated to the ExaLT Trial Office. The date and reason the participant withdraws consent (state 'reason unknown' if no reason provided) will be clearly documented in the participant's medical notes. Should the participant wish to withdraw from the mechanistic 'muscle' sub-study, they will still, unless otherwise specified remain in the ExaLT trial.

Participants found to be ineligible post randomisation should be followed up according to all trial processes and will still have their data analysed unless they explicitly change their level of participation.

The changes in levels of participation within the trial are categorised in the following ways:

No trial intervention: The participant would no longer like to receive the trial intervention, but is willing to be followed up in accordance with the schedule of assessments and if applicable using any central UK NHS bodies for long-term outcomes (i.e. the participant has agreed that data can be collected and used in the trial analysis).

No trial related follow-up: The participant does not wish to attend trial visits in accordance with the schedule of assessments, but is willing to be followed up at standard clinic visits and if applicable using any central UK NHS bodies for long-term outcomes (i.e., the participant has agreed that data can be collected at standard clinic visits and used in the trial analysis, including data collected as part of long-term outcomes).

No further data collection: The participant is not willing to be followed up in any way for the purposes of the trial AND does not wish for any further data to be collected (i.e., only data collected prior to any changes of levels in participation can be used in the trial analysis).

The details of changes of levels in participation within trial (date, reason and category of status change) will be clearly documented in the source documents (patient's medical notes and the Discontinuation CRF). The investigators can withdraw a participant from the trial, after consideration of the benefit:risk ratio, at any stage of the trial. Justifiable reasons for doing so include:

- Removal from the national LT waiting list, due to:
    - poor compliance with clinic/hospital visits and/or medical therapy (i.e. alcohol relapse)
    - terminal illness/palliation (i.e. HCC out of LT criteria, irreversible 'too' unwell for LT)
    - 'too' well for LT (i.e. liver disease has improved significantly that LT is not indicated)
    - participants request
- Technical grounds (e.g. patient moves away from trial area and can no longer meet the requirements of the trial protocol)
- Pregnancy (very *unlikely in this patient population*)
- Withdrawal of patient consent
- Unpredictable events (non-clinical or clinical): any event which at the discretion of the PI and/or CI makes further treatment inadvisable (i.e. incarceration)

All participants will be included in the analysis based on the intention to treat principle, either to the point of the end-point of the trial or to the point in which consent was withdrawn from participation in the trial.

9. ADVERSE EVENT REPORTING

9.19. Definitions

Table 8: Adverse event reporting definitions

Severity Definitions	Mild	Awareness of signs or symptoms that do not interfere with the participant's usual activity or are transient and resolved without treatment and with no sequelae.
	Moderate	A sign or symptom, which interferes with the participant's usual activity.
	Severe	Incapacity with inability to do work or perform usual activities.
Adverse Event	AE	Any untoward medical occurrence in a participant participating in the trial which does not necessarily have a causal relationship with the intervention received.
Related Event	RE	An event which resulted from the administration of any of the research procedures.
Serious Adverse Event	SAE	An untoward occurrence that: Results in death Is life-threatening* Requires hospitalisation or prolongation of existing hospitalisation Results in persistent or significant disability or incapacity Consists of a congenital anomaly/ birth defect Or is otherwise considered medically significant by the Investigator**
Unexpected Event	UE	The type of event that is not listed in the protocol as an expected occurrence.
Related and Unexpected Serious Adverse Event	N/A	A SAE that meets both the definition of a Related and Unexpected Event.

* The term life-threatening is defined as diseases or conditions where the likelihood of death is high unless the course of the disease is interrupted.

** Medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the participant or may require intervention to prevent one of the other outcomes listed in the definitions above.

9.20. Adverse event recording – general

The recording and reporting of Adverse Events (AEs) will be in accordance with the UK Policy Framework for Health and Social Care Research, the Principles of GCP and the requirements of the Health Research Authority (HRA). Definitions for AEs reporting are listed in **Table 8** in **Section 9.19**.

It is routine practice to record AEs in the participant's medical notes and it is also recommended that this includes the documentation of the assessment of severity and seriousness and also of causality (relatedness) in relation to the intervention(s) in accordance with the protocol.

9.21. Adverse event reporting in ExaLT

The study population have by definition a life-threatening liver disease that requires major curative LT surgery. Therefore, due to the nature of the severity of their disease and the symptom burden that accompanies the natural history of advanced liver disease, there are expected to be a very high number of Adverse Events (AEs) in this type of trial. For that reason, that the trial intervention (HBEP) has been assessed as being low risk to the study participants, the TMG has elected to only report Serious Adverse Events (SAEs).

The reporting period for SAEs in ExaLT will be from the day of randomisation (baseline visit 1) until the end of trial follow-up (visit 10). The safety profile for this trial population and interventions are well characterised so a strategy of targeted reporting of SAEs will not affect the safety of participants.

9.22. Serious Adverse Advents (SAE) reporting in ExaLT

For all SAEs, the PI or delegate must do one of the following:

- **Record safety reporting-exempt SAEs** in the medical notes but **not report** them to the trials office on an SAE form as per **Section 9.22.26**.

- **Report SAEs to the ExaLT Trial Office in a non-expedited manner**. This can only be done for the pre-defined subset of SAEs as per **Record safety reporting-exempt SAEs** in the medical notes but **not report** them to the trials office on an SAE form as per Section 9.22.26.

- **Report SAEs to the ExaLT Trial Office in a non-expedited manner**. This can only be done for the pre-defined subset of SAEs as per Section 9.22.27.

- **Report SAEs to the ExaLT Trial Office in an expedited manner** (within 24 hours of the site research team becoming aware of the event). All SAEs not covered by the above 2 categories must be reported as per **Section 9.23** Expedited SAE Reporting process.

Note: when an SAE occurs at the same hospital at which the participant is receiving trial intervention or is being followed up for trial purposes, processes must be in place to make the trial team at the hospital aware of any SAEs, regardless of which department first becomes aware of the event, in an expedited manner.

9.22.26. SAEs not requiring reporting to the ExaLT Trial Office

At whatever time they occur during an individual's participation, from the baseline visit to end of participant follow-up (visit 10), the following are not considered to be critical to evaluations of the safety of the trial:

- Hospital admissions that last less than 24 hours (e.g. symptomatic anaemia requiring no emergency intervention)
- Pre-planned hospitalisation (e.g. elective paracentesis or post-LT elective biliary drain removal)

All events which meet the definition of serious must be recorded in the participant notes, including the causality and severity, throughout the participant's time on trial, including follow-up, but for trial purposes these events do not require reporting on the SAE Form. Such events are "safety reporting exempt".

9.22.27. SAEs requiring non-expedited reporting to the ExaLT Trial Office

Where the safety profile is well established, the causal relationship between the intervention (or the participant's underlying condition), and the SAE, may be known. That is, such events are protocol-defined as "expected" (see Section 9.23.30 Assessment of expectedness of a related SAE by the CI).

Such events should still be recorded by the trial team in the participant's notes and reported to the ExaLT Trial Office on the follow-up CRF but they do not require expedited reporting (immediately on the site becoming aware of the event) since the assessment of expectedness for the specified events has been pre-defined. These include:

- **Pre-LT:**
    - admission to hospital due to hepatorenal syndrome/AKI, severe hepatic encephalopathy, variceal haemorrhage requiring OGD +/- banding, serious fall/musculoskeletal injury, cardiac or cerebrovascular event (STEMI/NSTEMI/angina/arrhythmia/CVA/cerebral haemorrhage), sepsis/infection as a consequence of SBP and/or pneumonia).

- **Post-LT:**
    - jaundice
    - confusion/delirium
    - fever/night sweats
    - severe gastrointestinal symptoms (vomiting/diarrhoea/nausea/loss of appetite)
    - significant fall/injury due to physical frailty
    - biliary stricture/anastomosis
    - bile leak
    - wound dehiscence
    - new onset ascites (requiring LVP, admission)
    - abdominal collection (requiring drainage/intravenous antibiotics),
    - haematoma/bleeding (requiring surgical/radiological intervention)
    - anaemia requiring blood transfusion
    - AKI (requiring renal replacement therapy)
    - ileus/bowel obstruction
    - re-laparotomy
    - portal vein thrombosis
    - hepatic artery (or conduit) thrombosis
    - bacteraemia requiring intravenous antibiotics
    - hyperglycaemia requiring insulin infusion
    - infection (pneumonia; cholangitis; urinary tract; wound; peritonitis; CNS; CMV),
    - acute rejection (biopsy proven*)
        - *mild, moderate or severe
        - pulsed steroids
        - T-cell or antibody-mediated rejection

9.22.28. SAEs requiring expedited reporting to the ExaLT Trial Office

All SAEs not listed in **sections 9.22.26** and **9.22.27** must be reported to the ExaLT Trial Office on a trial specific SAE form within 24 hours of the site research team becoming aware of the event. Examples include:

- Death
- Re-transplantation
- Multi-system organ failure requiring ICU support

9.23. Expedited SAE Reporting process

On becoming aware that a participant has experienced a SAE which requires expedited reporting the PI or delegate should report the SAE to their own Trust in accordance with local practice and to the ExaLT Trial Office.

To report an expedited SAE to the ExaLT Trial Office, the PI or delegate must complete, date and sign the trial-specific SAE form together with any other relevant, appropriately pseudoanonymised reports.

Data should be submitted to the ExaLT Trial Office using the information below in accordance with the timelines given in **Section 9.22.27** and **9.22.28**.

Non-expedited SAEs should be reported on a follow-up CRF.

To report a SAE, send the SAE Form to:
ExaLT@trials.bham.ac.uk

Where a SAE form has been completed by someone other than the PI initially, the original SAE form must be countersigned by the PI to confirm agreement with the causality and severity assessments.

On receipt of an SAE form, the ExaLT Trial Office will allocate each SAE a unique reference number and notify the site via email to the site as proof of receipt. The site and the ExaLT Trial Office should ensure that the SAE reference number is quoted on all correspondence and follow-up reports regarding the SAE and filed with the SAE in the ISF.

If the site has not received confirmation of receipt of the SAE or if the SAE has not been assigned a unique SAE identification number within 1 working day of reporting, the site should contact the ExaLT Trial Office.

9.23.29. Assessment of causality of a related SAE

When completing the SAE form, the PI (or, throughout this section, a medically qualified delegate) will be asked to define the nature of the seriousness and causality (relatedness; see **Table 9**) of the event.

In defining the causality the PI must consider if any concomitant events or medications may have contributed to the event and, where this is so, these events or medications should be reported on the SAE form. It is not necessary to report concomitant events or medications which did not contribute to the event.

As per **Table 9**, all events considered to be 'possibly', 'probably', or 'definitely' related to the intervention will be reported by the trial office as 'related'; all events considered at site to be 'unlikely' or 'unrelated' to the intervention will be reported by the trials office as 'unrelated'. The same categorisation should be used when describing AEs and protocol-exempt SAEs in the source data.

On receipt of an SAE form, the ExaLT Trial Office will forward it, with the unique reference number, to the CI or delegate who will independently* review the causality of the SAE. A SAE judged by the PI or CI or delegate to have a reasonable causal relationship ("Related" as per Table 9**: Categories of causality**) with the intervention will be regarded as a related SAE. The severity and causality assessment given by the PI will not be downgraded by the CI or delegate. If the CI or delegate disagrees with the PI's causality assessment, the opinion of both parties will be documented, and where the event requires further reporting, the opinion will be provided with the report.

*Where the CI is also the reporting PI an independent clinical causality review will be performed.

Table 9: Categories of causality

Category	Definition	Causality
Definitely	There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out.	Related
Probably	There is evidence to suggest a causal relationship, and the influence of other factors is unlikely.	
Possibly	There is some evidence to suggest a causal relationship. However, the influence of other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events or medication)	
Unlikely	There is little evidence to suggest there is a causal relationship. There is another reasonable explanation for the event (e.g., the participant's clinical condition, other concomitant events or medication).	Unrelated
Not related	There is no evidence of any causal relationship.	

9.23.30. Assessment of expectedness of a related SAE by the CI

The CI or delegate(s) will also assess all related SAEs for expectedness with reference to the criteria in **Table 10**.

Table 10: Categories of expectedness

Category	Definition
Expected	An adverse event that is consistent with known information about the trial related procedures or that is clearly defined in the relevant safety information. For the purposes of the ExaLT Trial, **Section 10** of the approved ExaLT protocol will be used as the reference safety information.
Unexpected	An adverse event that is <u>not</u> consistent with known information about the trial related procedures.

If the event is unexpected (i.e. it is not defined in the protocol as an expected event) it will be classified as a related and unexpected SAE.

The CI will undertake review of all related SAEs and may request further information from the clinical team at site for any given event(s) to assist in this.

9.23.31. Provision of SAE follow-up information

Following reporting of an SAE for a participant, the participant should be followed up until resolution or stabilisation of the event. Follow-up information should be provided using the SAE reference number provided by the ExaLT Trial Office. Once the SAE has been resolved, all critical follow-up information has been received and the paperwork is complete, a copy of the final version of the completed SAE form must be submitted to the Trial Office and the original kept in the ISF.

9.24. Reporting SAEs to third parties

The independent Data Monitoring Committee (DMC) may review any SAEs at their meetings.

The ExALT Trial Office will submit a progress report to the REC, UoB Research Governance Team (RGT) annually starting 12 months after the date of the favourable opinion was given. An electronic copy should be emailed to the REC within 30 days of the end of the reporting period. The Trial Office will report all events categorised as Unexpected and Related SAEs to the Research Ethics Committee (REC) and RGT within 15 days of being notified.

Details of all Unexpected and Related SAEs, and any other safety issue which arises during the course of the trial will be reported to the PIs. A copy of any such correspondence should be filed in the ISF and Trial Master File (TMF).

9.25. Urgent Safety Measures

If any urgent safety measures are taken, the Trial Office shall immediately, and in any event no later than 3 days from the date the measures are taken, give written notice to the REC of the measures taken and the reason why they have been taken.

9.26. Follow-up of pregnancy outcomes for potential SAEs

In the highly unlikely event that a participant was to become pregnant from date of consent until the end of the intervention period, the participant would be withdrawn from the intervention. We would however ask the participant if they were willing to continue being followed-up (data collection only).

The low risk (and general) nature of the intervention means that we would not follow-up the pregnancy or resulting offspring for SAEs.

10. DATA HANDLING AND RECORD KEEPING

10.27. Source data

Source data is defined as all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. In order to allow for the accurate reconstruction of the trial and clinical management of participants, source data will be accessible and maintained.

Some data variables may be entered directly onto the CRF; these are clearly identified and detailed in **Table 11**.

Table 11: Source data in ExaLT

Data	**Source**
Participant Reported Outcomes (i.e. trial questionnaires)	The original participant-completed CRF is the source and will be kept with the participant's trial record at site, whilst copies will be provided to the trial office.
Lab results	The original lab report (which may be electronic) is the source and will be kept and maintained, in line with normal local practice. Information will be transcribed onto CRFs.
Imaging (i.e. muscle USS)	The source is the original imaging usually as an electronic file. Data may be supplied to the ExaLT Trial Office as a password-protected, pseudoanonymised, copy of the electronic file, or as an interpretation of the imaging provided on a CRF. Where data is interpreted, the CRF onto which it is transcribed becomes the source. Copy of the CRF should be provided to the ExaLT trial office.
Physical function/physical activity data (i.e. CPET, accelerometer)	The source is the original test out-puts which will usually be provided as an electronic file. Data may be supplied to the ExaLT Trial Office as a password-protected, pseudoanonymised, copy of the electronic file, or as an interpretation of the test provided on a CRF. Where data is interpreted, the CRF onto which it is transcribed becomes the source. Copy of the CRF should be provided to the ExaLT Trial Office.
Clinical event data	The original clinical annotation is the source document. This may be found on clinical correspondence, or electronic or paper participant records. Clinical events reported by the participant, either in or out of clinic (e.g. telephone calls), must be documented in the source documents.
Recruitment	The original record of the randomisation is the source. It is held on BCTU servers as part of the randomisation and data entry system.
Withdrawal	Where a participant expresses a wish to withdraw, the conversation must be recorded in the source documents.

6.8.2

10.28. Case Report Form (CRF) completion

The CRFs will include (but will NOT be limited to) the following Forms (see **Table 12**).

Table 12: Case report forms in ExaLT trial.

Form Name	**Schedule for submission**
Randomisation form	At the point of randomisation
Baseline and follow-up CRFs including participant reported outcome measures (i.e. DASI; SF-36v2; 3 x behavioural questionnaires). These include visits 1-10, in addition to Day of LT CRF and inpatient admission post-LT CRF.	As soon as possible after each follow-up assessment time point
Mechanistic 'muscle' sub-study CRF	As soon as possible after each sub-study assessment timepoint (visit 1, 2 and 9)
SAE form	If expedited: emailed within 24 hours of site research team becoming aware of event If non-expedited: collected in the follow-up CRFs and sent (submitted soon as possible after each follow-up assessment time point)
Pregnancy notification form	As soon as possible after becoming aware of participant's pregnancy
Change of status form	As soon as possible after the point of reduced participation or death

A CRF should be completed for each individual participant.

In all cases it remains the responsibility of the PI to ensure that the CRF has been completed correctly and that the data are accurate. The signature of the PI or delegate will evidence this. The Site Signature & Delegation Log will identify all those personnel with responsibilities for data collection.

The delegated staff completing the CRF should ensure the accuracy, completeness and timeliness of the data reported. This will be evidenced by signing and dating the CRF.

Data reported on each CRF will be consistent with the source data and any discrepancies will be explained. All missing and ambiguous data will be queried. Staff delegated to complete CRFs will be trained to adhere to the ExALT Trial Protocol.

The following guidance applies to data and partial data:

- Only CRFs provided by the Trial Office should be used.

- Original completed CRFs or true copies should be sent to the ExaLT Trial Office with copies or originals filed in the ISF.
- Entries should be made in dark ink and must be legible.
- Any errors should be crossed out with a single stroke, the correction inserted and the change initialled and dated.
- Time format – all times should be in accordance with the 24 hour clock
- Rounding conventions – rounding should be to the nearest whole number: If the number you are rounding is followed by 5, 6, 7, 8, or 9, round the number up. E.g. 3.8 rounded to the nearest whole number is 4. If the number you are rounding is followed by 1, 2, 3 or 4, round the number down. E.g. 3.4 rounded to the nearest whole number is 3
- Trial-specific interpretation of data fields – where guidance is needed additional information will be supplied
- Entry requirements for concomitant medications (generic or brand names) – generic names should be used where possible
- Missing/incomplete data – should be clearly indicated – all blank fields will be queried by the ExaLT Trial Office
- Repeat laboratory tests – the data used to inform clinical decisions should always be supplied. If a test is repeated it is either to confirm or clarify a previous reading. Confirmatory tests should use the original test values.
- Protocol and GCP non-compliances should be reported to the ExaLT Trial Office upon discovery.

10.29. Participant completed questionnaires

Participant completed questionnaires will be administered by a member of the research team at site and will be completed by the participant, during their visit. A member of the research team will check the questionnaires for missing data, whilst the participant is still in attendance. If missing data of the questionnaires are identified, the participant will be given the opportunity to complete the questionnaire without any external input from the research team.

10.30. Data management

Processes will be employed to facilitate the accuracy and completeness of the data included in the final report. These processes will be detailed in the trial specific data management plan and include the processes of data entry, data queries.

Missing and ambiguous data will be queried using a data clarification system in line with the ExaLT data management plan and will focus on data required for trial outcome analysis and safety reporting. Single data entry with central monitoring will be employed.

With the exception of the randomisation system, (where data will be entered by staff at site). ExaLT Trial Office staff at BCTU will transcribe data from completed paper CRFs to an online database. The system will include data validations to improve data quality (e.g. to prevent nonsensical dates or numerical values). Changes to the data, on the system, will be made by ExaLT Trial Office staff and will

be documented and attributable. Again with the exception of the randomisation system, site staff will not have access to alter data on the online database but will be given a 'read-only view' of the database.

Site staff will be given unique log-in usernames and passwords to use the online randomisation system. These unique log-in details must not be shared with other staff and in no circumstances should staff at sites access the trial database using another person's login details. The ExaLT Trial Office will be unable to edit data in the randomisation system.

There will be no self-evident corrections to data made by the central ExaLT Trial Office staff.

10.31. Data security

UoB has policies in place, which are designed to protect the security, accuracy, integrity and confidentiality of Personal Data. The trial will be registered with the Data Protection Officer at UoB and will hold data in accordance with the Data Protection Act (2018 and subsequent amendments). The Trial Office has arrangements in place for the secure storage and processing of the trial data which comply with UoB policies.

The Trial Database System incorporates the following security countermeasures:
Physical security measures: restricted access to the building, supervised onsite repairs and storages of back-up tapes/disks are stored in a fire-proof safe.
Logical measures for access control and privilege management: including restricted accessibility, access controlled servers, separate controls of non-identifiable data.
Network security measures: including site firewalls, antivirus software and separate secure network protected hosting.
System management: the system will be developed by the Programming Team at the Trial Office, and will be implemented and maintained by the Programming Team.
System design: the system will comprise of a database and a data entry application with firewalls, restricted access, encryption and role based security controls.
Operational processes: the data will be processed and stored within BCTU.
System audit: The system will benefit from the following internal/external audit arrangements:

- Internal audit of the system
- Periodic IT risk assessment

Data Protection Registration: UoB's Data Protection Registration number is Z6195856.

10.32. Archiving

It is the responsibility of the PI to ensure all essential trial documentation and source documents (e.g., signed ICFs, Investigator Site Files, participants' hospital notes, CRFs) at their site are securely retained for the contractual period. Archiving will be authorised by BCTU on behalf of UoB following submission of the end of trial report. No documents should be destroyed without prior approval from the BCTU Director or their delegate.

The TMF will be stored at BCTU for at least 3 years after the end of the trial. Long-term offsite data archiving facilities will be considered for storage after this time; data will be stored securely and confidentially for at least 25 years. BCTU has standard processes for both hard copy and computer database legacy archiving.

11. QUALITY CONTROL AND QUALITY ASSURANCE

11.33. Site set-up and initiation

All PIs will be asked to sign the necessary agreements including a site signature and delegation log between the PI and the ExaLT Trial Office and supply a current CV and GCP certificate. All members of the site research team are required to sign the site signature and delegation log, which details which tasks have been delegated to them by the PI. The site signature and delegation log should be kept up to date by the PI. It is the PI's responsibility to inform the ExaLT Trial Office of any changes in the site research team.

Prior to commencing recruitment, each recruiting site will undergo a process of site initiation, either a meeting or a tele/videoconference, at which key members of the site research team are required to attend, covering aspects of the trial design, protocol procedures, adverse event reporting, collection and reporting of data and record keeping. Sites will be provided with an ISF containing essential documentation, instructions, and other documentation required for the conduct of the trial.

11.34. Monitoring

The central and on-site monitoring requirements for this trial have been developed in conjunction with the trial specific risk assessment and are documented in the trial specific monitoring plan.

11.34.32. On-site monitoring

For this trial, all sites will be monitored in accordance with the trial risk assessment and monitoring plan. Any monitoring activities will be reported to the ExaLT Trial Office and any issues noted will be followed up to resolution. Additional on-site monitoring visits may be triggered. PIs and site research teams will allow the ExaLT Trial Office staff access to source documents as requested. The monitoring will be conducted by BCTU/UoB staff.

11.34.33. Central monitoring

The ExaLT Trial Office will check received ICFs and CRFs for compliance with the protocol, data consistency, missing data and timing at a frequency and intensity determined by the data management plan. Sites will be sent DCFs requesting missing data or clarification of inconsistencies or discrepancies.

11.35. Audit and inspection

The PI (or delegate) will permit trial-related monitoring, audits, ethical review, and regulatory inspection(s) at their site and provide direct access to source data/documents. The PI (or delegate) will comply with these visits and any required follow-up. Sites are also requested to notify the ExaLT Trial Office of any relevant inspections or local audits.

11.36. Notification of Serious Breaches

The sponsor is responsible for notifying the REC of any serious breach of the conditions and principles of GCP in connection with that trial or of the protocol relating to that trial. Sites are therefore requested to notify the ExaLT Trial Office of any suspected trial-related serious breach of GCP and/or the trial protocol as soon as they become aware of them. Where the ExaLT Trial Office is investigating whether or not a serious breach has occurred, sites are also requested to co-operate with the Trial Office in providing sufficient information to report the breach to the REC where required and in undertaking any corrective and/or preventive action.

Sites may be suspended from further recruitment in the event of serious and persistent non-compliance with the protocol and/or GCP, and/or poor recruitment.

12. END OF TRIAL DEFINITION

The end of trial will be the date of the last data capture including resolution of DCFs. This will allow sufficient time for the completion of protocol procedures, data collection and input and data cleaning. The ExaLT Trial Office will notify the REC and the Sponsor within 90 days of the end of trial. Where the trial has terminated early, the Trial Office will notify the REC within 15 days of the end of trial. The Trial Office will provide the REC and the Sponsor with a summary of the clinical trial report within 12 months of the end of trial.

Ethical approval for the mechanistic ‘muscle’ sub-study will be granted as part of the main ExaLT protocol. Therefore, the date of last data capture (i.e. final visit [visit 10; 48-weeks post-LT] final patients; FPFV) will include the final processing of all samples (including the sub-study specialist biomarkers), as specified in the protocol.

13. STATISTICAL CONSIDERATIONS

13.37. Sample size

The mean PCS of the SF-36v2 QoL survey for patients with advanced liver disease or on the LT waiting list is approximately 39-42, with a standard deviation [SD] ranging from 8 to 24.(402, 412, 415, 416) Previous studies have indicated that LT alone improves PCS by +4 points (≈10%) compared to pre-LT.

In contrast, small studies post-LT have highlighted that basic, supervised exercise interventions improve the PCS by +8-9 points (≈20%). However, no studies to date have incorporated a pre- and post-LT exercise programme, with the addition of theory-based motivational support.

Hence for the sample size calculation we are proposing a +4 point (10%) improvement in the control arm and +12 point (30%) improvement in the experimental arm. Therefore, a meaningful clinically important difference [MCID] of 8 points with a SD of 16 will be used. The MCID is the smallest change in an outcome sufficiently important to influence management and is crucial for designing and interpreting comparative effectiveness trials. A MCID of +8 in QoL (using SF-36v2 questionnaires) has been previously reported in patients who rated their health as "excellent" or "very good" after abdominal surgery.(417) Members of the LT PPI group stated that improvement differences in PCS (SF-36v2) of up to 20% between the control and experimental treatment arms, would imply that the treatment has had a significant impact on the patients' QoL.

So with MCID of 8 points and SD of 16, 90% power and a type I error rate of 5%, using the standard method of difference in means (2-sided), a total of 172 participants will be required. NHS data reports that approx. 30-35% of patients on the waiting list for LT will not have their transplant within 1-year of randomisation (NHSBT database 2015 to 2020). Hence the sample size needs to be inflated to account for this and other possible dropouts. Adjusting for a 35% attrition/drop-out rate, a total of 266 participants (133 per group) will need to be recruited.

This sample size is predicted to be large enough to measure the impact of the intervention on the rate of surgical complications (CCI) post LT (i.e. key secondary outcome measure). The mean CCI post LT in European centres is approximately 40 (range 0-100). A 25% reduction in CCI with intervention versus the control (10-point i.e. 40 vs 30 by 6 months post transplantation) is considered as clinically meaningful improvement. With a total of 172 participants (before inflating for any attrition/drop-outs), we estimate that there will be approximately 74-87% power (alpha 5%, 2-sided test, SD 25) to detect a mean CCI difference of 10-12 points between the intervention versus control. We feel these are realistic, yet conservative estimates of power, as simulation methods (Stata 16) revealed higher power for non-parametric data – which is very possible with CCI post-LT.

13.38. Analysis of outcomes

A separate Statistical Analysis Plan will be produced and will provide a more comprehensive description of the planned statistical analyses. A brief outline of the planned analyses is given below.

The primary comparison groups will be composed of those randomised to intervention group versus those randomised to control group. Analyses will be based on the intention to treat (ITT) principle, i.e., all participants will be analysed in the intervention group to which they were randomised irrespective of adherence to randomised intervention or protocol deviations. However in the first instance, for the primary outcome and any relevant secondary outcomes, analysis will be based on the modified ITT set, with modified ITT set being patients that have had a LT.

For all major outcomes, summary statistics and differences between groups (e.g. mean differences, relative risks, hazard ratios, etc.) will be presented with 95% confidence interval from two-sided tests. Analyses will be adjusted for the minimisation variables and baseline scores (where appropriate). There will be no adjustment for multiple testing.

13.38.34. Primary outcome(s)

The primary outcome is the PCS from the SF-36v2 QoL at 6 months (24-weeks) post-LT. The SF36v2 QoL questionnaire will be administered at baseline for all randomised patients, during the pre-LT period (which will be variable between patients depending on how early they have their LT) and then at set intervals following their LT (i.e. at 6, 12, 24 and 48 weeks post LT). The data for this outcome is continuous and therefore will be summarised at each time-point (pre and post-LT) using the mean and standard deviation along with minimum and maximum values with respect to the intervention arms and overall.

Modified ITT set analysis

For the analysis of the primary outcome, initially the data collected at baseline and after LT will be considered in the first instance. Difference between group means and associated 95% CI at the primary time points (i.e. 24 weeks post-LT) will be estimated through the use of a repeated measures, mixed-effects linear regression model. Data collected at assessment times for baseline and the post-LT time points up to 24 weeks will be included. Data collected at 48-weeks post LT will not be included. Parameters allowing for participant, intervention arm, baseline score, time and the randomisation minimisation variables will be included (all as fixed effects). Time will be assumed to be a categorical (fixed) variable. To allow for a varying treatment effect over time, a time by treatment interaction parameter will be included in the model. Estimates of the mean differences between groups at the relevant time-points will be estimated from the model including this interaction parameter.

ITT set analysis

Now given there is a possibility that some patients that are randomised may not end up having a LT within 1 year of being randomised, they are initially excluded from the primary outcome analysis. However to ensure we account for all randomised patients, we will complete a secondary ITT analysis for the primary outcome which will include all randomised patients regardless of having had a LT.

This analysis will in the first instance only include data for the PCS of SF36v2 collected at baseline and for the pre-LT time-points only (excluding any data collected post-LT). Given patients will have a LT at different time-points (with some possibly never having a LT within 1 year of being randomised), we will explore this analysis using a joint model approach. This method of analysis will jointly fit the time to event (i.e. LT) data with the longitudinal PCS data collected at pre-LT time-points for all randomised patients. If there is no evidence of a significant difference between the groups with respect to time to transplant, then no further analyses will be undertaken and the results from the primary outcome analysis based on the modified ITT set will be considered as the main result for primary outcome.

However, if there is a significant difference between the study groups from the joint model, with respect to time to transplant, then further exploratory analysis will assist in interpreting the results

from the primary analysis. This will include imputing the SF-36v2 (by use of pattern mixture models to account for missing data not at random) to give an unbiased estimate from randomisation.

13.38.35. Secondary outcomes

The secondary outcomes are a combination of continuous, time-to-event and categorical data.

Continuous outcomes:

The secondary outcomes that are continuous data (e.g. CCI, MCS, DASI, LFI, length of ICU and hospital stay) will be analysed using the same analysis methods as described for the primary outcome modified ITT set.

Time-to-event outcomes:

The secondary outcomes that are time-to-event data (e.g. mortality) will be analysed using survival analysis methods. Kaplan-Meier survival curves will be constructed for visual presentation of time-to-event comparisons. A Cox proportional hazard model will be fitted, and results will be expressed as the hazard ratio with 95% confidence intervals.

Categorical outcomes:

The secondary outcome, LFI, can be summarised as continuous data (*as above*) or categorised into robust, pre-frail, or frail. The data for this outcome is summarised in 3 orderly categories; 1 = "≤3.2 (robust)", 2 = "3.2-4.5 (pre-frail)", 3 = ">4.5 (frail)". The analysis for this outcome will be conducted using a multilevel mixed-effects ordered logistic regression model and results will be expressed as odds ratio with 95% confidence intervals.

13.38.36. Planned subgroup analyses

Subgroup analyses will be limited to the same variables used in the minimisation algorithm (see Section 5 – ENROLMENT, RANDOMISATION and BLINDING) and performed on the primary outcome only. The effects of these subgroups will be examined by including an intervention group by subgroup interaction parameter in the regression model, which will be presented alongside the effect estimate and 95% confidence interval within subgroups. The results of subgroup analyses will be treated with caution and will be used for the purposes of hypothesis generation only.

13.38.37. Missing data and sensitivity analyses

Every attempt will be made to collect full follow-up data on all study participants; it is thus anticipated that missing data will be minimal. Participants with missing primary outcome data will not be included in the primary analysis in the first instance. This however presents a risk of bias, and so sensitivity analyses will be undertaken to assess the possible impact of the risk. In brief, this will include using multiple imputation with chained equations to impute any missing data.

We have also described the possible additional sensitivity analysis to account for missing data not at random for the primary outcome using pattern mixture models based on the results of the joint model taking into account time to LT and data for the primary outcome pre-LT (see primary outcome ITT set analysis).

Further sensitivity analysis will include a Complier Average Causal Effects (CACE) analysis for the primary outcome.

Full details will be included in the Statistical Analysis Plan.

13.38.38. Planned final analyses

The primary analysis for the trial will occur once:

- The last randomised patient has had their LT and their 24-weeks follow-up assessment post LT completed, OR
- When the last randomised patient has not had their LT within 1 year of being randomised and once all corresponding outcome data has been entered onto the trial database and validated as being ready for analysis.

14. SUB-STUDY: Mechanistic 'Muscle' sub-study (n=100)

The main aim of the 'muscle' sub-study is to undertake a detailed evaluation of the biological and physiological mechanisms that may underlie any exercised-induced improvements in clinical outcomes, including QoL and physical function/frailty. A better understanding of how exercise works (i.e. on the muscular and cardiopulmonary systems) will guide future studies in terms of exercise dose-responses ('frequency', 'intensity', 'duration') that are required in patients with end-stage liver disease to maximise the efficiency and longevity of LT.

The objectives of the 'muscle' sub-study are:

1. To calculate the 'dose' of exercise (frequency, intensity, duration) completed before (after 6-weeks intervention) and after LT (after 25-72 weeks intervention, depending on the timing of LT).
2. To determine if 'dose' of exercise achieved before and after LT is associated with changes in:
    a. QoL (PCS, MCS)
    b. physical frailty (LFI and its 3 components)
    c. cardiopulmonary fitness (DASI, CPET, 6MWT)
    d. muscle mass/thickness (quadricep ultrasound)
3. To investigate if the home-based exercise programme (HBEP) improves the following before and after LT:
    a. muscle mass/thickness (quadricep ultrasound)
    b. cardiopulmonary fitness (CPET, 6MWT)
    c. serological markers of oxidative stress/muscle inflammation (specialist biomarkers)

   and whether these improvements are associated with clinical measures of physical frailty (LFI) and QoL (PCS, MCS).

A sub-group of 100 participants (from a total of 266 patients enrolled in the ExaLT trial) will be recruited to the mechanistic 'muscle' sub-study. Participants will be recruited continuously on a

voluntary basis at both trial sites, until the target of 100 is achieved. Participants will have to provide written consent for the sub-study at the same time as providing consent for the main ExaLT study. The 'muscle' sub-study will contain the same proportion of participants in group 1 (n=50, exercise/motivation programme) and group 2 (n=50, control arm) as randomisation for the ExaLT trial will be stratified for participation in the 'muscle' sub-study (in addition to age, UKELD, gender and trial site). In addition, the DMC will be able to review (based on annual reports) that the baseline characteristics of the muscle sub-study population are representative of the main ExaLT trial.

At any stage between randomisation and 24 weeks post-LT (end-of-treatment [EOT]), a patient may withdraw consent from being a participant in the 'muscle' sub-group study, without necessarily giving a reason and without any personal disadvantage. The details of withdrawal will be clearly documented and communicated to the Trials Office. The date and reason the patient withdraws consent (state 'reason unknown' if no reason provided) will be clearly documented in the patient's medical notes. By withdrawing from the 'muscle' sub-study, unless specified, the patient will continue to be a participant for the remainder of the ExaLT trial, as this will not impact on the primary outcome measure (QoL).

After randomisation participants who have consented for the 'muscle' sub-study will undergo the following baseline investigations (*in addition to the ExaLT trial baseline investigations at visit 1; within 3 days*) prior to starting the study intervention (or control):

- CPET to determine standard measures such as anaerobic threshold and peak oxygen consumption (i.e. integrated response to the physiological stress of maximal exercise)
- Quadricep muscle ultrasound to assess skeletal muscle thickness.
- Venous blood sampling to assess the following specialist biomarkers:
- Common measures of oxidative stress: Total redox status, malonyldialdehyde, Myeloperoxidase, 4-Hydroxynonenal.
- Serum antioxidant capacity: catalase, glutathione peroxidase and superoxide dismutase.
- A profile of key myokines, including: interleukin(IL)-6, IL-10, IL-15, Irisin, leukaemia inhibitory factor, and secreted protein acidic and rich in cysteine (SPARC)
- Tumour necrosis factor alpha (TNF-α) (not a myokine, but an inflammatory marker).

These investigations will then be repeated after 6 weeks of study intervention (pre-LT visit 2) and 24 weeks after LT (post-LT visit 9; end of intervention). The baseline (visit 1) to 6 week (visit 2) pre-LT datasets will determine the short-term effect of the study intervention whilst on the LT waiting list. In the event that the participant undergoes LT prior to visit 2 (i.e. between weeks 0 to 6; unpredictable timing), the investigations will not be repeated until post-LT visit 9 (end of intervention). The post-LT dataset (visit 9) will determine the longer-term effect (i.e. prehabilitation and 24-weeks rehabilitation post-LT) of the study intervention on muscle, inflammation and cardiopulmonary fitness, alongside the main ExaLT trial primary and secondary outcome measures. Throughout the 'muscle' sub-study the control arm will provide the bench mark for the investigations performed on the pre-LT waiting list and 24-weeks after the LT. A full standard operating procedure (SOP) will be produced for this sub-study.

15. TRIAL ORGANISATIONAL STRUCTURE

15.39. Sponsor

The Sponsor for this trial is University of Birmingham (UoB).

15.40. Coordinating centre

The trial-coordinating centre (ExaLT Trial Office) is Birmingham Clinical Trials Unit (BCTU), based at UoB.

15.41. Trial Management Group (TMG)

The Trial Management Group (TMG) comprises of individuals responsible for the day-to-day management of the trial: the CI, Co-CI, PIs, Co-applicants, Trial Statistician, Trial Manager and Data Manager. The role of the group is to monitor all aspects of the conduct and progress of the trial, ensure that the protocol is adhered to and take appropriate action to safeguard participants and the quality of the trial itself. The TMG will meet sufficiently frequently to fulfil its function.

15.42. Trial Steering Committee (TSC)

A Trial Steering Committee (TSC), comprising independent and non-independent members, will be established for the ExaLT Trial and will meet as required depending on the needs of the trial. Membership and duties/responsibilities are outlined in the TSC Charter. In summary, the role of the TSC is to provide oversight of the trial. The TSC will monitor trial progress and conduct, and provide advice on scientific credibility. The TSC will consider and act, as appropriate, upon the recommendations of the Data Monitoring Committee (DMC). The TSC will operate in accordance with a trial specific TSC Charter.

15.43. Data Monitoring Committee (DMC)

The role of the independent DMC is to monitor the trial data, and make recommendations to the TSC on whether there are any ethical or safety reasons as to why the trial should not continue or whether it needs to be modified. To this end, data on safety outcomes and (where appropriate) primary and major secondary outcomes will be supplied to the DMC during the trial. Reports will be supplied in confidence. The DMC will operate in accordance with a trial specific DMC Charter which will define the membership, roles and responsibilities of the DMC. The DMC will meet at least annually as a minimum. Additional meetings may be called if needed e.g., recruitment is faster than anticipated or a safety issue is identified.

15.44. Finance

The research costs of the ExaLT trial are funded by the National Institute of Health Research (NIHR) Efficacy and Mechanism Evaluation Programme (Ref: NIHR129318) awarded to Dr. Matthew Armstrong, University of Birmingham. The trial has been designed to minimise extra 'service support' costs for participating hospitals as far as possible. Additional costs, service support costs and excess treatment costs associated with the trial, e.g., gaining consent, are estimated in the Statement of Activities. These costs should be met by accessing the Trust's Support for Science budget via the Local Comprehensive Research Network.

16. ETHICAL CONSIDERATIONS

The ExaLT trial will be conducted in accordance with the UK Policy Framework for Health and Social Care Research and applicable UK Acts of Parliament and Statutory Instruments (and relevant subsequent amendments), which include Data Protection Act 2018; Human Tissue Act 2004; Mental Capacity Act 2005; and the Principles of GCP. The protocol will be submitted to and approved by the REC prior to the start of the trial. Before any participants are randomised into the trial, the PI at each site is required to obtain the necessary local approval.

It is the responsibility of the PI to ensure that all subsequent amendments gain the necessary local approval. This does not affect the individual clinicians' responsibility to take immediate action if thought necessary to protect the health and interest of individual participants.

17. DATA PROTECTION AND CONFIDENTIALITY

Personal data and sensitive personal data recorded on all documents will be regarded as strictly confidential and will be handled and stored in accordance with the Data Protection Act 2018 (and subsequent amendments). Personal data categories that will be collected and analysed include name, date of birth, NHS number and primary/secondary NHS healthcare records (including past medical history, GP practice, drug history).

Participants will only be identified by their **3 digit unique trial identification number** and **initials** on routine correspondence with the ExaLT BCTU Trial Office. The following personal identifiable data (PID) will be collected on the CRFs:

Table 13: PID captured in the ExaLT trial.

Form name	**PID**
Randomisation form	Trial number, participant's full name, date of birth, gender and NHS number
Baseline and follow-up CRFs including participant reported outcome measures (i.e. DASI; SF-36v2; 3 x behavioural questionnaires). These include visits 1-10, in addition to day of LT CRF and inpatient admission post-LT CRF Mechanistic 'muscle' sub-study CRF Change of status form	Trial number and initials
SAE form Pregnancy notification form	Trial number and partial date of birth

Participants will acknowledge the transfer and storage of their informed consent form to the ExaLT Trial Office. This will be used to perform central monitoring of the consent process. Participants will acknowledge the transfer of their personal data for the purpose of medical research to BCTU at UoB. Participants will acknowledge the transfer of their personal data to BCTU at UoB, who will be processing data on behalf of the trial.

In the case of specific issues and/or queries from the regulatory authorities, it will be necessary to have access to the complete trial records. Representatives of the ExaLT Trial Office and sponsor (UoB) may be required to have access to participants' notes for quality assurance purposes, but participants should be reassured that their confidentiality will be respected at all times. The ExaLT Trial Office will maintain the confidentiality of all participant data and will not disclose information by which participants may be identified to any third party.

18. FINANCIAL AND OTHER COMPETING INTERESTS

There are no financial or other competing interests related to the results of this trial. Members of the TSC and DMC are required to provide declarations on potential competing interests as part of their membership of the committees. Authors are similarly required to provide declarations at the time of submission to publishers.

19. INSURANCE AND INDEMNITY

UoB has in place clinical trials indemnity coverage for this trial which provides cover to UoB for harm which comes about through the University's, or its staff's, negligence in relation to the design or

management of the trial and may alternatively, and at UoB's discretion provide cover for non-negligent harm to participants.

With respect to the conduct of the trial at site and other clinical care of the patient, responsibility for the care of the patients remains with the NHS organisation (QEUHB or RFH) responsible for the clinical site and is therefore indemnified through the NHS Litigation Authority.

20. POST-TRIAL CARE

In keeping with the Declaration of Helsinki 2013, all trial participants will be followed up in their routine NHS post-LT clinics and will receive the standard of healthcare in place at the time. If the participant has any ongoing additional healthcare needs at the end of the trial (i.e. disability, mental health illness) they will be referred onto the relevant specialist (i.e. physiotherapist, social care worker, psychiatrist) by the clinical/research team (i.e. PI or local clinician). There will be NHS trust funding (QEUHB, RFH) to prescribe the physiotherapist delivered 'home-based exercise and theory-based motivation support programme' in the future if the trial proves that the intervention is safe and efficacious (i.e. meets the primary end-point).

21. ACCESS TO FINAL DATASET

The final dataset will be available to members of the Trial Management group (TMG) and co-applicant group who need access to the data to undertake the final analyses.

Requests for data generated during this study will be considered by BCTU. Data will typically be available 6 months after the primary publication unless it is not possible to share the data (for example: the trial results are to be used as part of a regulatory submission, the release of the data is subject to the approval of a third party who withholds their consent, or BCTU is not the controller of the data).

Only scientifically sound proposals from appropriately qualified Research Groups will be considered for data sharing. The request will be reviewed by the BCTU Data Sharing Committee in discussion with the CI and, where appropriate (or in absence of the CI) any of the following: the Trial Sponsor, the relevant TMG, and independent TSC.

A formal Data Sharing Agreement (DSA) may be required between respective organisations once release of the data is approved and before data can be released. Data will be fully de-identified (anonymised) unless the DSA covers transfer of participant identifiable information. Any data transfer will use a secure and encrypted method.

22. PUBLICATION PLAN

Outputs from the ExaLT trial will be submitted for publication in peer reviewed journals and the findings of the trial will be made public. Manuscripts will be prepared by the writing group as defined in the trial publication plan. Manuscripts should be submitted to the TMG in a timely fashion and in advance of being submitted for publication to allow time for review. The participants will be provided with a lay written summary of the outcome of the trial, alongside provision of the publication.

In all publications, authors should acknowledge that the trial was performed with the support of the NIHR, University of Birmingham and BCTU. Intellectual property rights will be addressed in the site agreement between Sponsor and site.

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