THE EVALUATION OF SARCOPENIA IN CHRONIC LIVER DISEASE

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A thesis submitted to the University of Birmingham for the degree of

DOCTOR OF PHILOSOPHY

Institute of inflammation and Ageing

College of Medical and Dental Sciences

University of Birmingham

August 2022

ABSTRACT

End stage liver disease (ESLD) has a significant impact on the homeostasis of muscle, with observed reductions in muscle mass, performance and function due to an ongoing catabolic state. This study carried out a multi-modal assessment, including MRI, muscle functional measures and analysis of serum and muscle biopsies, to evaluate and phenotype sarcopenia and frailty in those with ESLD and to determine the mechanisms driving compromised muscle health. The study recruited 53 patients with ESLD, 39 of whom completed the multi-modal assessments, and 18 age and sex matched healthy controls. The ESLD cohort had a median age of 57.5 (IQR 50.0-61.3) years with 61.9% male predominance and the controls had a median age of 51.5 (IQR 33.0-63.3) years with 61.1% who were male . The disease aetiologies were 47.9% alcohol related disease, 14.3% NAFLD and 33.3% autoimmune disease. The findings highlighted that reduced muscle mass, quality and function (as measured by strength and performance) were reduced in those with ESLD compared to healthy controls, that when evaluating changes in muscle composition, muscle mass, quality and function should all be considered in those with ESLD. This study found that quadriceps measures of muscle mass from MRI and ultrasound, correlated to the commonly used standard of L3 SMI, supporting its future use as a functionally relevant muscle group. Further, the results showed that muscle mass, quality and function were impacted by ascites, hepatic encephalopathy, aetiology of ESLD, age and sex, supporting the consideration of these variables when evaluating muscle health in ESLD. Finally, the muscle phenotype in patients with ESLD was broken down in to those with adequate muscle mass and function, those with adequate mass but inadequate function and those with inadequate mass and function. Comparing these distinct phenotypes revealed clear differences in the mechanisms potentially driving muscle wasting in ESLD at a molecular and transcriptomic level; notably altered adrenal steroids, mitochondrial

dysfunction, cellular senescence, altered regulation of protein synthesis and pro-inflammatory pathways were identified as differential influences on muscle phenotype. Taken together, these data support the need for a multi-modal assessment to evaluate sarcopenia and frailty in ESLD with targets for future research highlighted.

For Karam and Jaya, to remind them that anything is possible.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank all the participants who volunteered to take part in this study, without who none of this research would have been possible.

Secondly, I would like sincerely thank my supervisors, Professor Janet Lord, Dr Matthew Armstrong and Dr Ahmed Elsharkawy for their unwavering support, continued guidance and encouragement over the past 5 years. Their mentorship has been instrumental in completing this work. I would also like to thank Dr Jonny Quinlan, (my other 'unofficial' supervisor) and Felicity Williams for their friendship, teamwork and support during this time.

I would like to thank the team within the National Institute for Health and Care Research (NIHR) Biomedical Research Centre (BRC) Birmingham Sarcopenia theme, including Dr Sophie Allen, Dr Tom Nicholson, Prof Leigh Breen, Prof Gareth Lavery, and Prof Simon Jones, for their ongoing support and collaboration. I would like to thank Prof Carolyn Greig and Prof Phil Newsome, in particular, for their mentorship and guidance over the years.

I would like to thank the team within the National Institute for Health and Care Research (NIHR) Clinical Research (CRF) and Inflammation Research Facilities (IRF) and Queen Elizabeth Hospital Birmingham Liver Unit, for their assistance and support during this study. In addition I would like to thank the team within the Lord research group and Institute of Inflammation and Ageing for their help with this work. I would like to especially thanks Dr Jon Hazeldine and Dr Kirsty McGee for their endless patience and help within the lab.

Finally, I would like to thank all my family and friends from their support, kindness and encouragement during this period of my life. I would like to thank my parents, who instilled the work ethic and drive I needed to complete this thesis, and my family (Harjeet, Bhupinder, Jason, Karam and Jaya) for their understanding and encouragement. Finally, I would like to sincerely thank my friends, Frances, Bonnie and Bal, for their unwavering kindness, their friendship, and for always reminding me, during the hardest of times, that there is light at the end of the tunnel.

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ABBREVATIONS

αKG	alpha ketoglutarate
β-NGF	nerve growth factor
11βHSD1	11β-hydroxysteroid dehydrogenase type 1
24,25(OH)	24,25-dihydroxyvitamin D3
25OHD2	25-hydroxyvitamin D2
25OHD3	25-hydroxyvitamin D_3
3-epi-25OHD3	3-epi-25-hydroxy Vitamin D3
6MWT	six minute walk test
AA	amino acids
AASLD	american association for the study of liver
ACh	acetylcholine
ACSA	anatomical cross sectional area
ADP	adenosine diphosphate
AIH	autoimmune hepatitis
AKT	protein kinase B
ALP	alkaline phosphatase
ALT	alanine transaminase
AMA	anti mitochondrial antibody
AMP	adenosine monophosphate
AMPK	adenosine monophosphate activated protein kinase
ANA	anti-nuclear antibody
ANOVA	analysis of variance
APTT	activated partial thromboplastin time
ArLD	alcohol related liver disease
ASMA	anti smooth muscle antibody

AST	aspartate aminotransferase
ATP	adenosine triphosphate
BIA	bio impedance analysis
BMI	body mass index
BMR	basal metabolic rate
CLD	chronic liver disease
CLD Q	chronic liver disease questionnaire
CMV	cytomegalovirus),
CRP	c reactive protein
CT	computerised tomography
CT -SMI	computerised tomography skeletal muscle index
CTACK	CC chemokine, cutaneous T cell-attracting chemokine
DASI	duke activity status index
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone Sulphate
DNA	adenosine monophosphate (AMP)
DXA	dual X ray absorptiometry
EASL	european association for the study of liver disease
EBV	epstein barr virus
eGFR	estimated glomerular filtration rate),
EIF 2	eukaryotic Initiation Factor 2
ELISA	enzyme linked immunosorbent assay
ESLD	end stage liver disease
ESLD	end stage liver disease
ESPEN	european society for clinical nutrition and metabolism
EWGSOP	european working group on sarcopenia in older people

FGF basic	fibroblast growth factor
FLEXIT	fitness, life enhancement, and exercise in liver transplantation
FNDC5	fibronectin type III domain containing protein 5
FoxO	fork head box protein O
FXR	farnesoid X receptor
G-CSF	granulocyte colony-stimulating factor
GGM-CSF	granulocyte-macrophage colony-stimulating factor
GGT	gamma glutamyltransferase
GH	growth hormone
GIP	glucose-dependent insulinotropic polypeptide
GLP-1	glucagon-like peptide-1 agonists,
GRO-α	growth regulated alpha
HAV	hepatitis A virus
Hb	haemoglobulin
HbA1c	glycolated haemoglobin
HBV	hepatitis B virus
HCV	hepatitis C virus
HE	hepatic encephalopathy
HFE	hereditary haemochromatosis
HGF	hepatocyte growth factor
HGS	handgrip strength
HIV	human immunodeficiency virus
HVPG	hepatic venous pressure gradient
ICU	intensive care unit
IFN-α2	interferon alpha 2
IFN-γ	Interferon gamma

IG	immunoglobulin
IGF -1	insulin like growth factor
IL-10	interleukin-10
IL-12	interleukin-12
IL-13	interleukin-13
IL-15	interleukin-15
IL-16	interleukin-16
IL-17a	interleukin-17a (
IL-18	interleukin 18
IL-1ra	interleukin-1 receptor antagonist
IL-1α	interleukin-1 alpha (
IL-1β	interleukin-1 beta
IL-2	interleukin-2
IL-2Rα	interleukin-2 receptor alpha
IL-3	interleukin-3
IL-4	interleukin-4
IL-5	interleukin-5
IL-6	interleukin-6
IL-7	interleukin-7
IL-8	interleukin-8
IL-9	interleukin-9 g
IMAT	intermuscular adipose tissue
INR	international normalised ratio
IP-10	Interferon gamma-induced protein 10 (
KPS	karnofsky performance status
L3 SMI	3rd lumbar vertebral skeletal muscle index

LC-MS/MS	liquid chromatography-mass spectrometry
LFI	liver frailty index
LIF	leukaemia inhibitory factor
LKM	liver kidney antibody
LT	liver transplantation
M-CSF	macrophage colony-stimulating factor
MAC	mid arm circumference
MAF-Bx	muscle atrophy F box or atrogin-1
MAMC	midarm muscle circumference
MCP-1	monocyte chemoattractant protein-1 (),
MCP-3	monocyte chemotactic protein-3
MCV	mean corpuscular volume
MELD	model for endstage liver disease
MET	metabolic rate equivalent
MIF	macrophage migration inhibitory factor
MIG	monokine induced by interferon-gamma
MIP 1α	macrophage Inflammatory Proteins
MIP 1β	macrophage Inflammatory Proteins
MPB	muscle protein breakdown
MPS	muscle protein synthesis
MRI	magnetic resonance imaging
MTBE	tert-butyl methyl ether
MTOR	direct mammalian target of rapamycin
MTORC1	direct mammalian target of rapamycin complex 1
MurF-1	muscle ring finger 1
MUST	malnutrition universal screening tool

NAFLD	non-alcoholic fatty liver disease
NF-κβ	nuclear factor kappa-light-chain-enhancer of activated B cells
NHSBT	national health service blood and transplant
NMJ	neuromuscular junction
p70S6K	ribosomal protein S6 kinase beta-1
PA	physical activity
PAI-1	plasminogen activator inhibitor-1
PBC	primary biliary cholangitis
PCSA	physiological cross sectional area
PDGF-BB	platelet-derived growth factor-BB
PGC1α	peroxisome proliferator-activated receptor gamma coactivator 1 alpha
PHT	portal hypertension
PI3K	phosphatidylinositol 3-kinase
PIS	patient information leaflet
PSC	primary sclerosing cholangitis
PT	prothrombin time
RANTES	regulated upon activation, normal T cell expressed and presumably secreted
RCT	randomised control trial
RFH – NPT	royal Free Hospital – Nutritional Prioritising Tool
RNA	ribonucleic acid
ROS	reactive oxidative species
RPE	rating of perceived exertion
RXR	retinoid X receptor
SARS COV- 2	severe acute respiratory syndrome coronavirus 2
SCF	stem cell factor
SCGF-β	stem cell growth factor-beta

SDF-1 α	stromal cell-derived factor 1
SF36	short form 36 questionnaire
SLE	supported liquid–liquid extraction plate
SMI	skeletal muscle index
SPPB	short physical performance battery
TCA	tricarboxylic acid cycle
TEE	total energy expenditure
TGFβ1	transforming growth factor beta 1
TNF- β	tumour necrosis factor beta
TNF-α	tumour necrosis factor alpha
TPMT	transverse psoas muscle thickness
TRAIL	tumour necrosis factor (TNF)-related apoptosis-inducing ligand
TSF	triceps skin fold thickness
UKELD	united kingdom model for end stage liver disease
UPS	ubiquitin proteasome system
US	ultrasound
VDR	vitamin D receptor
VEGF	vascular endothelial growth factor
VL	vastus lateralis
VO_{2max}	maximal aerobic capacity
WCC	white cell count

1. Chapter One: Introduction

1.1 Skeletal muscle physiology and structure

1.1.1 Skeletal muscle

Skeletal muscle is one of the three most significant muscle groupings within the human body and accounts for 40% of total body weight (1). It plays a fundamental role in posture, locomotion and motor function (2). More recently, it has also been recognised as an integral part of immune regulation (3) and energy metabolism (4). It is a voluntary muscle, and its action can be controlled actively and passively. It is maintained by the homeostasis of anabolic (protein synthesis) and catabolic (protein degradation) signalling (5, 6). Additionally it functions as an endocrine organ by secreting myokines such as interleukin 6 (IL-6) to regulate immune responses (7), and by storing amino acids (AA) and glycogen. Skeletal muscle undergoes constant remodelling and has significant plasticity as it adapts to both positive and negative stimuli (5, 6).

1.1.2 Skeletal muscle structure

Skeletal muscle is formed of skeletal muscle fibres, a neurovascular supply and connective tissue (Figure 1.1). The skeletal muscle fibres in conjunction with motor neurons form motor units. The smallest subunit of skeletal muscle begins with a myocyte, an elongated multinuclear cell. These fuse together to form a myofibril which then collect together to form an individual myofibre (or muscle fibre). Each myofibre contains the following: myofibrils (proteins), sarcoplasm, mitochondria, nuclei, sarcoplasmic reticulum (specialised smooth endoplasmic reticulum) and is surrounded by the sarcolemma (a plasma membrane specific

to skeletal muscle cells) (5, 8). Each myofibre is encased in a layer of connective tissue called the endomysium.

Myofibres are arranged in bundles called fascicles which are surrounded by the perimysium (middle layer of connective tissue). Each muscle itself is enclosed by epimysium (dense irregular connective tissue sheath) which allows muscular contraction whilst its structure remains intact and separates it from other viscera. These connective tissue layers are intertwined with collagen at the one end of the tendon, with the other fused with the periosteum layer of bone, allowing movement and contraction. In some cases, this epimysium is attached to aponeuroses or fascia (5, 9, 10).

Each myofibril consists of a sarcomere and thin and thick myofilaments. The arrangement of these thin and thick filaments provides the striated appearance of skeletal muscle. The thin filaments are comprised of actin protein chains with a myosin binding site and are associated with the regulatory proteins, troponin and tropomyosin, which control the exposure of actin binding sites for binding to myosin. The thick filaments are comprised of myosin protein complexes (2 heavy and 4 light chains of myosin). These filaments contain binding sites for actin and a binding site for ATP (11). The sarcomere is the contractile, functional unit of a myofibre, that lies between the actin and myosin filaments including the M and Z lines, and the A, H and I bands (Figure 1.1). It is the I and H bands which shorten with muscle contraction (11, 12).

1.1.3 Muscle physiology

The structure of the muscle allows for contraction and movement, a process triggered by the nervous system. Each muscle fibre is supplied by the axonal branch of a somatic motor neuron. Signalling from the neuron triggers a skeletal myofibre to contract; the thin actin filaments are pulled and slide past the thick myosin filaments within the sarcomere. It is the sarcomere that shortens whilst the filaments remain static in length (13).

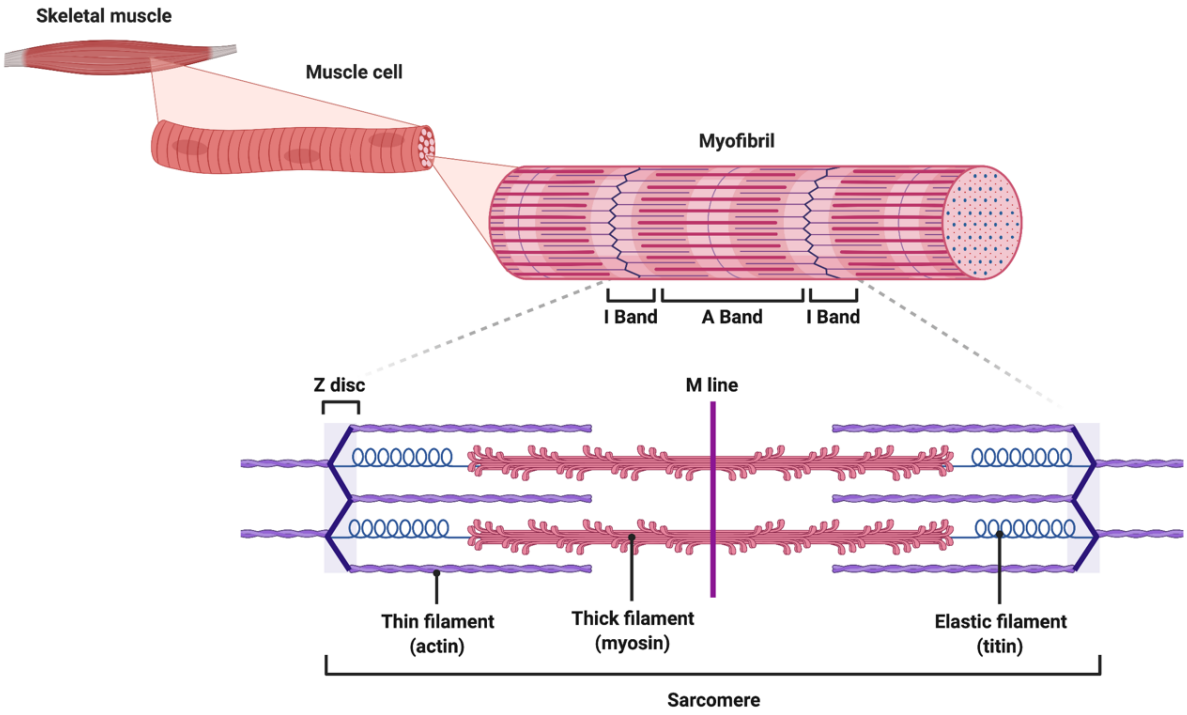

Figure 1-1 Structure of a myofibril:

Skeletal muscle is comprised of myofibrils. Each myofibril consists of a sarcomere which contains thin actin and thick myosin myofilaments, with an elastic titin filament. Their arrangement provides the striated appearance of skeletal muscle. There are three bands within the structure I, A, H; and two lines M and Z (aka disc). Figure created from Biorender.com

1.1.3.1 Muscle contraction and neuromuscular signalling

Muscle contraction is controlled by the Excitation Contraction Coupling process. This initial neural signalling occurs at the neuromuscular junction (NMJ), where the motor neuron terminal and myofibre meet (14) (Figure 1.2). Contraction of muscle fibre begins with the release of acetylcholine (ACh) from the motor neuron. ACh binds to the ACh receptors within the end plate of the sarcolemma and opens voltage gated sodium ion channels, initiating the excitation contraction coupling process. This allows depolarisation of the sarcolemma with positively charged sodium ions, which in turn trigger the action potential that depolarises the remainder of the membrane/sarcolemma. This further triggers calcium ion release from the sarcoplasmic reticulum into the sarcoplasm. The calcium ion influx into the sarcoplasm exposes the myosin binding sites on the actin filaments by shifting tropomyosin away from them. The currents are generated from ions moving through channel proteins on the sarcolemma. The myosin binds to the actin binding sites in a cross-bridge formation which is followed by the force/ power stroke of sliding of the actin filaments by the myosin filaments; this part of the process requires ATP (5, 11, 12, 14-16). The ACh within the synaptic cleft in the NMJ is degraded by acetylcholine esterase to prevent ACh rebinding to the receptors in the sarcolemma (5, 11, 12, 14, 15).

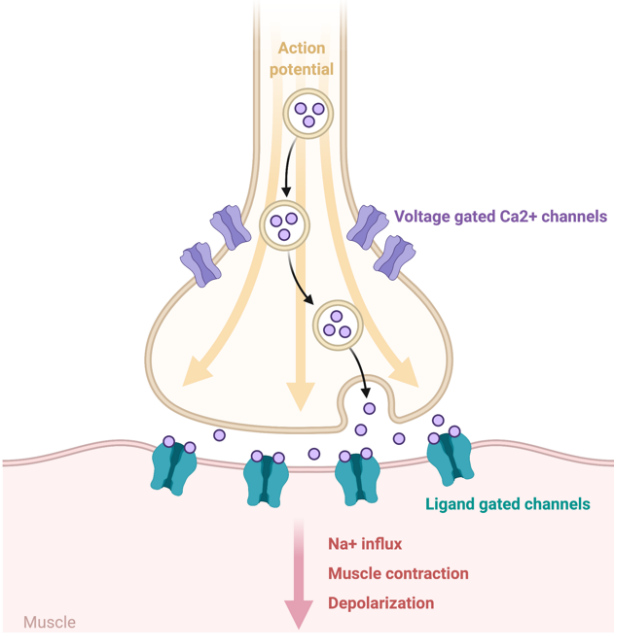

Figure 1-2 The neuromuscular junction

The NMJ is site at which the motor neuron terminal meets the myofibre, and is where the initial neural signalling occurs for muscular contraction, via the excitation contraction coupling process.
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Following contraction, filaments need to reset for further contraction, so need to detach, reset and attach etc which is termed cross bridge cycling, an ATP dependent process (5, 11, 12, 14, 15). ATP for skeletal muscle contraction is generated via the following process:

1. Creatine phosphate: Excess ATP can transfer the energy to creatine, forming creatine phosphate and adenosine diphosphate (ADP). This can be transferred back rapidly when ATP is required, using creatine kinase as the catalyst.
2. Glycolysis: This is an anaerobic process where glucose is catabolised to produce ATP, however at a slower rate compared to creatine phosphate. The glucose substrate can be obtained from blood glucose or from glycogen stored in the muscle. The breakdown of glucose produces 2 ATP and 2 pyruvic acid molecules which can be utilised for aerobic

metabolism. However when oxygen is not available, it is converted to lactic acid, which can be a contributing factor in muscular fatigue.

3. Aerobic respiration: This is a mitochondria driven process which requires glucose, pyruvic acid and fatty acids. It produces 36 ATP molecules. It requires a steady oxygen supply and skeletal muscle compensates by storing excess oxygen in myoglobin.

Muscle fatigue occurs when there is a disconnect between muscle contraction and it's signalling. The exact cause is not fully understood. However it is hypothesised that this may be due to reduced ATP reserves, the effects of lactic acid on enzyme and protein activity, and electrolyte imbalances in the cell membrane depolarisation, disturbing calcium regulation required for muscle contraction (5, 9).

1.1.3.2 Muscle tone and types of contraction

Muscle contraction involves muscle strength and tension. Muscle strength corresponds to the number of myofibrils and sarcomeres within each muscle fibre. The number of skeletal muscle fibres is genetically determined and remains unchanged. Muscle hypertrophy increases the production of myofibres and sarcomeres, increasing skeletal muscle bulk and mass, whereas the converse is true for muscle atrophy. Muscle tension corresponds to the force generated by muscle contraction and correlates to the length of the sarcomere, therefore as the sarcomere shortens, the muscle tension is increased (5, 9).

There are several types of muscular contractions: *Isotonic contraction*: the muscle tension (force) remains constant and a load is moved as the length of the muscle changes; *Concentric contraction*: the muscle shortens to move a load; *Eccentric contraction***:** occurs when muscle

tension is reduced, and the muscle lengthens and *Isometric contraction*: muscle produced tension without a change in muscle length (5, 9).

A single contraction is described as a muscle twitch. A twitch has three phases; latent period (where the action potential is propagated), a pre-contraction phase, a contraction phase (tension increases) and a relaxation phase (where tension decreases). Motor neurons can vary the rate at which action potentials are fired, modifying the degree of tension generated by the innervated muscle fibres, this is called the graded muscle response (5, 9, 12).

Muscle tone allows stabilisation of joints and maintenance of posture. Muscles are seldom completely flaccid and are usually contracted to some degree to produce muscle tone. The size of a motor unit (a single motor neuron and all the muscle fibres innervated by it) dictates its function (5, 9, 12).

1.1.3.3 Muscle fibre types

Skeletal muscle fibres can be classified by: 1) how fast the fibres contract relative to others and how quickly they produce cross bridge actions (i.e. fast versus slow); or 2) how the fibres generate ATP (i.e. oxidative or glycolytic) (5, 9). There are three types of muscle fibres, slow oxidative, fast oxidative and fast glycolytic fibres:

Type I: Slow twitch oxidative fibres, red (myoglobin), resistant to exhaustion, produce large amounts of ATP (aerobic).

Type IIa: Fast twitch oxidative fibres, large numbers of mitochondria and myoglobin, produce and metabolise ATP (aerobic and anaerobic).

Type IIb: Fast glycolytic fibres, white (low myoglobin), low, slow rate ATP production (anaerobic), fast breakdown (5, 9).

Oxidative fibres contain more mitochondria than glycolytic fibres, as the process of aerobic metabolism occurs in the mitochondria. They are able to contract for prolonged periods as they produce significant amounts ATP. They are smaller in diameter and as a result do not produce a large amount of tension. They also have an extensive blood supply, aiding oxygen delivery, and have large myoglobin stores which gives them a dark, red colour, also allowing oxygen storage. These fibres are optimised for stabilisation and maintenance of posture (5, 9).

Fast glycolytic fibres have a larger diameter, contain large volumes of glycogen to generate ATP. They have limited mitochondria and myoglobin, with a limited capillary supply as they do not rely on aerobic respiration (10, 14).

Fast oxidative fibres have characteristics of both fast-glycolytic fibres and slow oxidative fibres. They can produce high amounts of tension but do not fatigue quickly due to oxidative elements. They are used in an intermediary capacity for movements stronger than postural control but less energy consuming than an explosive intense movement (5). Muscle cells can change in terms of size of the myofibres (hypertrophy or atrophy), however new cells are rarely formed from resident stem cells (also known as satellite cells) in muscle growth (5).

1.1.3.4 Effect of exercise on muscle

Exercise can enhance angiogenesis of the muscle, improving oxygen supply and removing waste products. Suitability of skeletal muscle for endurance is determined by the proportion

of slow oxidative muscle fibres. Postural muscles need a greater proportion of slow oxidative fibres to fast glycolytic fibres to constantly maintain posture (5, 10). In endurance exercise (limited force but numerous repetitions), aerobic metabolism is used by slow oxidative muscle fibres as contractions are required over a long period of time. These slow fibres become more efficient as endurance exercise produces more mitochondria, which increases myoglobin synthesis, allowing improved excess oxygen storage and Increased ATP production. This in turn, increases the rate of aerobic metabolism (5, 9, 10).

Resistance exercise (where muscular contraction is opposed by a force to increase strength) in contrast targets fast glycolytic fibres and increases myofibril formation, increasing the diameter of muscle fibres, and induces muscle enlargement by adding structural protein. Muscles utilised in resistance exercise have a greater proportion of fast glycolytic fibres to slow oxidative. Muscle hypertrophy can be enhanced by increased protein intake in addition to resistance exercise. Resistance exercise also increases the connective tissues and improves tendon strength (as the force of muscle is transferred to the tendon it is attached to) (5, 9, 10, 17).

1.1.4 Regulation of muscle mass

Skeletal muscle has a structural plasticity which means it has the ability to alter its structural and functional properties in accordance with the environmental conditions imposed on it (13). This is driven by satellite cells, which are skeletal muscle stem cells located outside of the sarcolemma that are incorporated into skeletal muscle to facilitate protein synthesis. They are stimulated to grow by growth factors under stress and can partially regenerate muscle fibres, though to a limited extent. Their primary function is to help repair damage to muscle.

They are regulated by insulin like growth factor (IGF-1), IL-6 and myostatin, which play significant roles in muscle protein synthesis and homeostasis.

Muscle protein balance is maintained by the rate of muscle protein synthesis (MPS) and breakdown (MPB). MPS is regulated largely by the IGF1- phosphatidylinositol 3-kinase (PI3K) - protein kinase B (Akt) – mammalian target of rapamycin (mTOR) pathway whereas MPB is affected by two major pathways: 1. Ubiquitin proteasome system (UPS); 2. Calpain mediated protein cleavage (18). These are explained further below.

1.1.4.1 Regulation of muscle protein synthesis

In muscle hypertrophy, IGF-1 binding to its receptor activates the PI3K-AKt-mTOR pathway to promote MPS. AKt in turn deactivates the transcription factor Fork head box protein O (FoxO) preventing induction of atrogenes, muscle atrophy F box or atrogin-1 (MAFbx) and muscle ring finger 1 (MurF-1), reducing MPB (19). In muscle atrophy, reduced IGF-1 activity downregulates the PI3K/AKt pathway, allowing for upregulation and activation of FoxO and induction of MAFbx and MurF1 signalling, potentiating MPB. Pro-inflammatory cytokines such as IL-6 and tumour necrosis factor alpha (TNFα), dampen IGF-1 production and signalling, promoting atrophy (19). When the energy supply is low or deficient, AMP builds up in muscle activating AMP Kinase (AMPK) which inhibits the mTOR complex 1 (mTORC1), upregulates myostatin, activates FOXO and induces atrogenes to initiate MPB (19) (Figure 1.3) .

1.1.4.2 Regulation of muscle protein breakdown

Ubiquitin Proteasome Pathway

This pathway accounts for the majority of protein degradation (20). It is an ATP dependent pathway, in which a ubiquitin protein substrate binds to three ligase enzymes (E1, E2, and E3 ligases), and undergoes degradation by a 26S proteasome complex (18). Muscle specific E3 ligases, also known as atrogenes (MAFbx and MurF1) regulate these atrophic signals, leading to MPB.

Calpain mediated proteolysis

Calpains are a sub group of cysteine proteases mediated calcium dependent proteolysis of cytoskeletal and membrane proteins. Calcium dependent proteolysis has been involved in various types of muscular atrophies (21). Inflammation can cause oxidation of the protein involved in calcium transplant, which in turn results in impaired muscular contraction (22). Calpains are linked to myogenic cell proliferation and migration, thus oxidative stress can result in satellite cell dysfunction and impaired muscle regeneration (23).

1.1.4.3 Autophagy

Autophagy is the process of removing damaged and dysfunctional organelles within a cellular system, and is important in skeletal muscle homeostasis. The removal of damaged organelles includes mitochondria and is termed mitophagy. Autophagy occurs in response to cellular stress such as energy exhaustion and starvation, a frequent state in patients with chronic liver disease. It is also involved in the regulation of the production of inflammatory mediators in acute inflammation (24). It is hypothesised that dysregulation of autophagy may be driven by oxidative stress. Upregulation of the NF-κβ pathway can inhibit autophagy and promotes

production of pro-inflammatory cytokines, further enhancing MPB (25, 26). Within models of ageing, it has been shown that autophagy is impaired with reduced clearance and increased accumulation of waste products which can induce apoptosis and loss of cells. Reduced autophagy can also induce cellular senescence; these senescent cells are highly pro-inflammatory and release proteases such as matrix metalloproteases (27), which can contribute to muscle damage and poor function (28). Reduced mitophagy would also inhibit function in a tissue such as muscle which is very dependent on ATP generation and optimal mitochondrial function.

1.1.5 Myokines

Myokines are cytokines or peptides which are synthesised and secreted by myocytes in response to muscle contraction. They are an integral part of autocrine muscle regulation and have a systemic impact (29, 30). There are several important myokines:

*Myostatin***:** This was the first myokine identified in 1997 by Lee and colleagues (31) and is also known as growth differentiation factor 8. It is part of the transforming growth factor (TGF) beta protein family and is found in skeletal muscle predominantly, however there is some expression in cardiac muscle and adipose tissue. It decreases in response to exercise and inhibits MPS (29, 30).

Irisin: This is a cleaved form of fibronectin type III domain containing protein 5 (FNDC5) which is stimulated when peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC1α) is expressed (32). It increases thermogenesis (energy lost as heat) and regulation of energy by the conversion of white adipose to brown adipose tissue (32). These brown adipocytes contain more capillaries and supply tissues including muscle, with greater oxygen

and nutrients (32). Irisin and myostatin are inversely released from skeletal muscle following physical exercise, demonstrating a myogenic role for irisin (33).

IL-6: This is secreted from blood vessels in response to muscular contraction during exercise, when insulin activity is enhanced (34). IL-6 levels increase based on duration and intensity of the contraction. IL-6 increases insulin stimulated glucose removal, glucose uptake and fatty acid oxidation via adenosine monophosphate (AMP) activated protein kinase (AMPK) and P13K-AKT signalling pathways (35).

1.1.5.1 Energy Expenditure

In addition to its locomotive and contractile function, skeletal muscle has metabolic and homeostatic functions, and is involved in energy expenditure (36). Skeletal muscle, especially total lean muscle accounts for a significant amount of the body's basal metabolic rate (BMR), therefore any reduction in muscle mass, in turn reduces BMR. Total energy expenditure (TEE) is comprised of BMR, physical activity (PA) and dietary induced thermogenesis (DIT). BMR is the energy required for normal physiological function and it accounts for 50-70% of TEE. It is affected by weight, body composition e.g. fat free mass is more metabolically active than fat mass and expends more energy for the equivalent gram, age, gender and environment (37). Importantly, BMR is affected by nutritional status, disease, inflammation, hormonal status, drugs and psychological state. This is important in the context of chronic liver disease. Energy expenditure can be measured directly (through measurement of heat production) via direct calorimetry; or indirectly by measuring oxygen (O2) consumption via indirect calorimetry.

1.1.6 Physical Activity

Physical activity (PA) is defined as any movement which causes energy expenditure by contraction of skeletal muscle and accounts for 20-40% of TEE. PA can be measured through subjective assessment (via questionnaires or PA recall) or through an objective assessment such as pedometry or accelerometry. Pedometers detect vertical movement at the hip and can measure steps and distance travelled. They do not take into account the difference in a body habitus or physicality (38, 39). Accelerometry is a measure of acceleration in either a single or multiple directions; vertical ± triaxial ± mediolateral and antero-posterior directions. It records the movement in different time intervals which can then be analysed to calculate to formulate PA variables and sedentary time (37). PA is measured by frequency, duration, patterns, types of activity and intensity. Intensity is defined as metabolic rate equivalent (MET). This describes PA in terms of a person's resting oxygen uptake defined as a rate of consumption of 3.5ml of O2/min/kg of body weight. It can determine PA intensity within the ranges of light (1.5-2.9 METs); moderate (3.0-5.9 METs); and vigorous (≥6.0 METs) (37, 40, 41).

Sedentary behaviour is defined as any waking behaviour characterized by an energy expenditure ≤1.5 METs while in a sitting, reclining or lying posture. Sedentary behaviour can often be used to describe physical inactivity, however people can demonstrate sedentary behaviour yet be physically active at set periods in the day (42).

1.1.7 Nutrition

Skeletal muscle protein synthesis is stimulated by feeding (43). During basal, post absorptive and inflammatory states, the rate of MPB outweighs the rate of MPS. Nutritional intake

offsets this balance by providing dietary protein derived amino acids to stimulate MPS, and by insulin release in response to hyperglycaemia from nutritional intake, which suppresses MPB (44, 45). The rate of stimulation of MPS is dependent on the amount of protein consumed. However there is a plateau effect with MPS once more than a certain amount of protein is consumed at a single time point. A short interval between consumption is thus more effective at enhancing MPS (46). The degree of MPB suppression by insulin, whose release is triggered by the protein and carbohydrate consumed, is not as amount dependent (45, 47).

1.1.8 Exercise

Exercise is defined as a subset of physical activity that is planned, structured, and repetitive and has as a final or an intermediate objective the improvement or maintenance and conditioning the body. The aim is to maintain or improve physical fitness (48). Exercise increases muscle mass due to an increase in MPS which exceeds MPB post exercise, in keeping with an improved net protein balance (44). Both MPS and MPB are increased following exercise. During muscle contraction, there can be a slight decrease in MPS, which then rebounds with a significant increase in MPS (49). Exercising in a fasted state does differ as MPB exceeds MPS (50). Consuming food following exercise causes an increase in MPS which then results in a net muscle protein gain (45).

1.1.9 Ageing and muscle

Ageing is associated with compromised protein synthesis. The phenomenon of anabolic resistance increases with advanced age and is defined by a blunted response to the anabolic effects of amino acids and exercise and the anti-proteolytic effect of insulin on MPS and MPB. Anabolic resistance causes a gradual decline in muscle mass and function in older adults (46,

51) and results in the elderly having to ingest more protein or carry out a higher level of resistance exercise to achieve the same anabolic response as a young person (52). Age-related changes to muscle are further described in Section 2.

1.1.10 Sleep and muscle

Sleep has been implicated in muscle regulation. Murine models have shown that paradoxical sleep deprivation, a physical stressor, may mediate muscle atrophy by inducing a catabolic state with increased plasma corticosterone and reduced plasma testosterone (53). The effects of corticosterone and testosterone are further discussed in the mechanisms of sarcopenia in ESLD, section 5. Studies using healthy men, show that the rate of myofibrillar protein synthesis, which is a key variable in regulating skeletal muscle mass, is reduced, suggesting the sleep deprivation may contribute to muscle atrophy (54).

1.1.11 Psychological impact

Any affect mood and mental health will have an indirect impact on muscle regulation. Depressive symptoms often include reduced appetite, anhedonia resulting in reduced physical activity and commonly impaired sleep. As described above, changes to these lifestyle factors, will negatively impact muscle homeostasis by reducing anabolic stimuli and favouring MPB. Other examples include mania, where there can be significant weight loss and potential loss of muscle mass. Additionally, poor psychological wellbeing can modify behaviour, and consequently affect compliance and adherence with management, and lifestyle factors involved in supporting muscle maintenance. There are established associations between mental health conditions such as depression and fatigue (55) and the positive impact of physical activity on symptoms of anxiety and depression is well known (56). However

increasingly there may be a further association of these conditions e.g. depression and fatigue with an increased inflammatory activation of the immune system (57), thus muscle homeostasis may be directly affected by mental health.

1.2 Overview of Sarcopenia

1.2.1 Defining sarcopenia

Sarcopenia is a muscle wasting syndrome which is now increasingly recognised clinically. The term 'sarcopenia' is derived from the Greek language for 'sarx' meaning 'flesh' and 'penia' meaning 'poverty.' It was originally described to illustrate the process of skeletal muscle decline in an ageing population in 1989 by Rosenburg (58). In order to formulate a consensus, the European Working Group of Sarcopenia in Older People (EWGSOP) published the definition of sarcopenia as the presence of low muscle strength with low muscle mass. It is determined as severe when low physical performance is additionally detected (59). Furthermore, for a quantitative definition of sarcopenia, cut offs are taken as more than two standard deviations below the mean of a young healthy reference population (59). Sarcopenia is now increasingly recognised as it is associated with functional decline, physical disability and adverse impacts on health care costs, quality of life, morbidity and mortality (60).

With time, we have recognised the effect of other factors influencing sarcopenia, in the absence or addition to ageing, such as inflammation and chronic systemic disease states, this is termed as Secondary sarcopenia (58, 61). Furthermore, EWGSOP identified sub categories of acute and chronic sarcopenia (59). Acute sarcopenia is defined as sarcopenia lasting for less than 6 months whereas it can be defined as chronic when it lasts more than 6 months. Whilst acute sarcopenia is usually related to an acute insult such as injury or a sudden illness

(62), there is a recognition that chronic sarcopenia exists with chronic and progressive conditions (59). This thesis will attempt to phenotype sarcopenia in chronic liver disease, with a view to understanding some of the observations and associations seen.

The concept of sarcopenia is incorporated in several other syndromes such as frailty, malnutrition, and cachexia. It is important to understand how these differ as they can often be used interchangeably. They are defined below to highlight how the defining features of a sarcopenia diagnosis are incorporated into these terms:

Frailty: This is defined as a clinical state where there is enhanced vulnerability to developing increased dependency and/or mortality when exposed to a stressor. Fried *et al* (63) first described an operational criteria of frailty in 2001, comprising three out of the following; low grip strength, low energy, slowed walking/ gait speed, low physical activity +/- unintentional weight loss. This has evolved into a 'frailty index' which is argued to be a superior predictor of clinical outcomes due to a finer graded risk scale and incorporation of clinical inferences into the index (64). Disease specific frailty indices are also being developed such as the Liver Frailty Index (65).

Malnutrition: This is defined as referring to deficiencies, excesses, or imbalances in a person's intake of energy and/or nutrients (66). This incorporates both undernutrition and over nutritional states. There are several validated screening tools in use to evaluate malnutrition such as Malnutrition Universal Screening Tool (MUST). A criterion was formulated by using these screening tools to diagnose malnutrition by the Global Leadership Initiative on Malnutrition. This involved using phenotypic markers (non-intentional weight loss, low body mass index (BMI) or reduced muscle mass) and aetiological markers (reduced food intake /

assimilation or disease burden and inflammatory conditions) (67). One of each of these markers fulfils a criterion of malnutrition.

Cachexia: This is defined as a multifactorial syndrome with involuntary progressive weight loss due to reduction in skeletal muscle mass, with or without depletion of adipose tissue (68). It is often used in the context of cancer, where cancer cachexia is specifically characterised by systemic inflammation and metabolic changes, leading to progressive functional impairment (69). It incorporates nutritional assessment and measurement of muscle mass and body composition (70).

These terms all involve a form of muscle quality +/- strength compromise, hence why they are often used interchangeably. Therefore, many people with sarcopenia will also have malnutrition, cachexia and frailty.

1.2.2 Sarcopenia pathogenesis

The pathogenesis of sarcopenia is complex and only partially understood. It is driven by a multitude of factors and mechanisms which may differ dependent on the context in which sarcopenia develops. The most commonly described mechanisms stem from research in age-related sarcopenia (71), these include cellular senescence, satellite cell and mitochondrial dysfunction, neuromuscular dysfunction, and anabolic resistance. There several additional lifestyle factors such as poor nutritional intake, reduced physical activity and sleep which also play a role in developing and enhancing sarcopenia. They are discussed further below.

1.2.2.1 Cellular senescence:

This describes a state of permanent cell cycle arrest. It occurs in response to a number of stressors, such as telomere length shortening, oxidative stress, mitochondrial dysfunction and

DNA damage (72). This phenomenon of cellular senescence is particularly important in sarcopenia, when it occurs in satellite cells in skeletal muscle. As previously described, satellite cells play an integral role in regeneration of the muscle. Therefore, the development of cellular senescence halts muscular regeneration, growth and repair. To date most of the research showing cell senescence in muscle has been carried out in murine models (72) and research on this process in human skeletal muscle is lacking.

The impact of cell senescence extends beyond the impact on regenerative potential and relates to the secretory activity of these cells. The Senescence Associated Secretory Phenotype (SASP) describes the output of senescent cells; they secrete a variety of pro-inflammatory cytokines, proteases, chemokines, growth factors and extracellular vesicles (73). The different cell types which produce SASPs can have a beneficial or negative effect depending on the secrotome. For example senescent fibroblasts are important for wound healing (74). However, age-related senescent cells have a predominantly pro-inflammatory profile which contributes to increased systemic inflammation; which is termed 'inflammaging' (75). Research shows that inflammatory cytokines such as TNF-α, IL-6 and C-Reactive Protein (CRP) are elevated in older people and in sarcopenia (76-78). This 'inflammaging' can potentially drive skeletal muscle ageing and sarcopenia by inducing catabolic processes (72); these include the induction of 11βHSD1 which generates cortisol in the muscle itself (79).

1.2.2.2 Satellite cell dysfunction:

Satellite cells are activated to proliferate in skeletal muscle to facilitate muscle regeneration and repair (80). They are regulated by a number of growth factors including IL-6, IGF-1, myostatin (81) and myonuclear accretion (82). They are usually quiescent in a basal resting

state. Following damage, they proliferate and fuse with muscle fibres, in turn upregulating myogenic regulatory protein expression which is regulated by the transcription factor Pax 7 (18, 83, 84). With increasing age, the number of satellite cells present in muscle declines, in turn reducing the ability for skeletal muscle to regenerate and repair (85). Satellite cells are also negatively affected extrinsically by fibroblast growth factor (86), TGFβ1 (87), and myostatin (88) therefore resulting in reduced regenerative capacity which is observed in both ageing and chronic inflammation.

1.2.2.3 Mitochondrial dysfunction

Skeletal muscle has a dense network of mitochondria in order to carry out its function in physical activity. As demonstrated in ageing studies, damage to mitochondria causes an increase in reactive oxidative species (ROS) generation and a reduction in cellular energy, resulting in further mitochondrial dysfunction and damage to mitochondrial DNA which can eventually lead to cellular senescence as described above. In addition, damaged organelles including mitochondria are normally removed by mitophagy, but this process is compromised with ageing (89). Atrophy of the muscle can occur as a consequence of each of these changes (72, 90).

1.2.2.4 Muscle fibre type changes and Neuromuscular dysfunction

There is a change in muscle fibre composition and number with age as well as alteration to the neuromuscular junction structure with muscle wasting and sarcopenia. In particular there is a reduction in the size of muscle fibres and the number of muscle fibres in older adults compared to young, preferentially a type II fibre loss (91, 92). Other studies have demonstrated a loss of type I muscle fibres, in addition to an occurrence of mixed fibre types

in older participants which not observed in younger individuals (93), a mechanism which may also facilitate dysfunction. This alteration in the size, number of and type of muscle fibres, affects both muscle mass and quality. One of the causes of muscle fibre loss is thought to be a decrease in the number of motor units with age (91) with denervation and reduction of the axonal cell body size. This may promote dysregulated neuronal activation (94, 95) with a decreased capacity for re-innervation (95). An intact NMJ is required to support muscle fibres and loss of NMJ innervation leads to muscle fibre loss (96).

1.2.2.5 Anabolic resistance

This term refers to a phenomenon that occurs when there is an impaired rate of cellular anabolism in response to various anabolic stimuli such as branched chain amino acids, IGF-1, insulin and exercise, resulting in a blunted and dampened MPS response (52). Normally in response to anabolic stimuli, the mTOR pathway is activated and triggers transcriptional processes via the S6 kinase, enhancing ribosomal biogenesis and also resulting in inhibition of atrogene expression thus promoting muscle protein synthesis (52, 97-99). Studies have shown that there is a decrease in MPS in response to anabolic stimuli with ageing; this is postulated to be due to the inhibition of IGF 1 signalling, and diminished mTOR signalling in response to upstream activation of anabolic signals via PI3k-Akt pathway (52). In human studies, Cuthbertson et al (51) and others, have shown anabolic resistance in response to several stimuli such as BCAA (52), other AA (100) and exercise (101), with inflammation contributes to this phenomenon (52).

1.2.2.6 Hormonal influences

Sarcopenia is associated with hormonal changes such as loss of androgenic hormones and insulin resistance (102, 103). Ageing is associated with a decreased sensitivity to insulin, a decrease in growth hormone (GH), in IGF-1, and testosterone, and the decline of the resilience of the hypothalamic pituitary axis in the elderly (104), all promoting catabolism.

Insulin promotes muscle protein synthesis and has an anabolic effect via binding to the IGF-1 receptor and signalling through mTOR (105). Insulin resistance leads to higher levels of circulating insulin, impaired gluconeogenesis, reduced glycogen synthesis, increased free fatty acid uptake, increased lipolysis and altered triglyceride transport; all of which exacerbate proteolysis (106). Increased free fatty acids inhibit GH. GH inhibits myostatin, thus the low GH activity increases myostatin activity and muscle protein breakdown is promoted (107) (Figure 1.3).

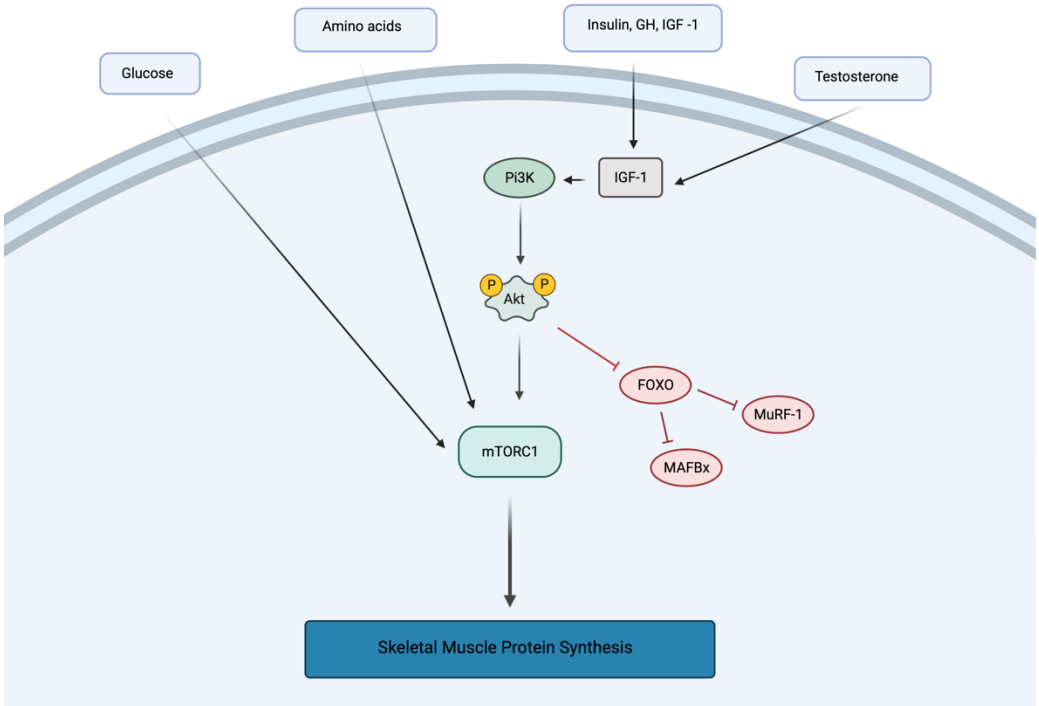


Figure 1-3: Regulation of muscle protein synthesis

MTORC1 is activated to stimulate protein synthesis via glucose (nutrient intake and glycolysis)), amino acids, and hormonal trigger, whilst maintaining inhibition of atrogene formation to inhibit muscle protein breakdown. GH (growth hormone), IGF-1 (insulin like growth factor), PI3K (phosphatidylinositol 3-kinase), Akt (protein kinase B), FOXO (forkhead box protein), mTORC1 (mammalian target of rapamycin), MAFBx (muscle atrophy F box or atrogin-1) and MurF-1(muscle ring finger 1). Created using Biorender.com.

Testosterone levels decline with age and are reduced in conditions such as chronic liver disease. In males, levels decrease by 1% per year, and in women, levels drop significantly from years 20 to 45 (108). Testosterone induces MPS (109) but can be modulated by different elements including genetics, exercise and nutrition (110). Testosterone supplementation attenuates decreases in muscle mass and hand grip strength, however this intervention is limited as it is also associated with significant side effects (110).

GH is also involved in the coordination the muscle growth. GH levels decline with increasing age, as does IGF-1 whose production is regulated by GH, termed somatopause (111). Therefore, stimulation of the Akt and mTOR pathways, are reduced. Additionally, the adipokine Ghrelin stimulates the production of GH and inhibits production of pro-inflammatory cytokines IL-6 and TNFα. Low ghrelin levels are observed in older adults, contributing to age related sarcopenia (108).

1.2.2.7 Inflammation

Inflammatory cytokines such as TNFα, IFNγ, and IL-6 promote muscle proteolysis through two main pathways; P38 MAPK and the NF-κB pathways which stimulate the induction of atrogenes MuRF-1 and MAFbx (95). Inflammatory mediators may also contribute to the dysregulation of autophagy by oxidative stress stimulation, resulting in reduced removal of

damaged organelles or protein and increase in cellular stress and further production of pro-inflammatory cytokines (25, 26). Research in murine and human studies suggest the pro-inflammatory cytokine TNFα also has an effect on satellite cell activity and differentiation by reducing myoblast determination protein 1 expression and regeneration of muscles (112-115). TNF*α* is also a primary signal inducing apoptosis in muscle (108) and directly upregulates the NF-κβ pathway promoting MuRF-1 expression (116). Upregulated TNF*α* expression, in inflammation, appears to also enhance apoptosis through increased mitochondrial dysfunction resulting in a loss of muscle fibres and atrophy (117). IL-6 specifically, can activate UPS, E3 ligase and proteasome activity, induce insulin resistance which suppresses Akt and mTOR activity, promoting muscle protein degradation (79).

As mentioned earlier cytokines such as TNFα and IL-6 can also induce generation of cortisol in muscle via induction of 11βHSD1, promoting MPB. This activity also increases with age (118). Glucocorticoids are also released during the inflammatory response and in addition to their catabolic effects they also inhibit GH secretion, thus reducing IGF-1 activity, and upregulating the myostatin activity with impacts on MPS (108).

1.2.2.8 Nutrition

Protein intake is one of the major anabolic stimuli for MPS, providing the essential amino acids required. The optimal recommended dietary protein intake is between 1.0 - 1.2 g/kg (body weight)/day to prevent sarcopenia (119). Lower intake of food, as often observed in older adults, leads to weight loss and potential muscle loss. The deficiencies in the overall diet and/or specific nutrients that result from lower intake of food are postulated to contribute to sarcopenia (120). Nutrition thus has an important role to play in both the prevention and

management of sarcopenia (121). There have been many studies including several RCTs, studying the role of protein and nutritional supplementation in sarcopenia. There is a critical role for the dietary intake of protein alone and in combination with exercise in preventing sarcopenia; this combination gives the optimal effect (120).

With regards to micronutrients, vitamin D is the most studied. Vitamin D is thought to have many important roles within skeletal muscle including maintaining contractile excitability via intracellular calcium, muscle stem cell proliferation and differentiation and the consequential maintenance of muscle function (122). Unsurprisingly, a deficiency or even an insufficiency of vitamin D is correlated with the risk of diseases including sarcopenia, cardiovascular disease, obesity and cancer (123). According to the UK Scientific Advisory Committee on Nutrition (SACN), vitamin D deficiency is classified as serum levels of 25-hydroxyvitamin D (25-OH-D) below <25 nmol/L. However, others suggest that deficiency is serum levels of 25-OH-D below 25–50 nmol/L, with sufficiency >75 nmol/L and hence insufficiency in the region of 51–74 nmol/L (124).

Vitamin D deficiency is associated with sarcopenia in older people (122) Whilst the specific mechanisms whereby vitamin D deficiency contributes to muscle loss are unclear; it is likely that the vitamin D receptor (VDR) plays a role via changes in anabolic signalling, muscle protein synthesis and translational efficacy (125, 126). Aside from the direct actions on skeletal muscle, the VDR is also known to impact mitochondrial function, whereby depletion of the VDR results in reduced oxidative phosphorylation output (127). In addition, mitochondrial dysfunction can result in the increased production of reactive oxygen species, which are known to have a negative impact on skeletal muscle and hence contribute to

sarcopenia (90). Vitamin D supplementation in adults improves muscle strength in pre-sarcopenic elderly patients (128), healthy 18- to 40-year-olds (129) and post-menopausal cohorts (130).

1.2.2.9 Physical activity and sedentary behaviour

Physical activity is the second major anabolic stimulus for MPS. Improving physical activity from inactive people to becoming physically active can add 1.3–3.7 years to an individual's life, showing general health benefits (131). Studies from community-dwelling older adults indicate that physical activity (PA) and sedentary time were associated with sarcopenia or components of sarcopenia (low muscle mass, low grip strength, or slow gait speed) (132, 133). Meier et al showed that 5000 steps/day was significantly associated with odds of having low muscle mass and slow gait speed indicating that being active was associated with higher muscle mass and faster gait speed (132). A systematic review and meta-analysis by Steffl *et al*, (134) showed that PA protects against sarcopenia, with a reduced risk of acquiring sarcopenia in later life (121).

There is also direct evidence to support the use of exercise in reducing sarcopenia. Aerobic exercise has been shown to increase MPS in older individuals. A Cochrane review of 121 randomised control trials in older people also demonstrated that resistance exercise training (RET) two to three times per week caused improvements in muscle mass and strength measures (135). RET studies demonstrated that muscle cross sectional area improved by 11% in addition to increased strength (>100%) following a 12 week period of RET in older males (136). Muscle contraction by resistance exercise increases the overall rate of muscle protein turnover, but does so disproportionately in favour of MPS (137).

1.2.2.10 Sleep

Sleep is an important factor for both muscle regeneration and hormonal homeostasis. Sleep duration and quality are both compromised with ageing and often also in chronic inflammatory disease. It has been shown that with adverse sleep patterns and sleep disturbances, sleep deprivation leads to an imbalance between catabolic and anabolic hormone secretions; thus, sarcopenia may be causally related to poor sleep (103, 138).

Both hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axes are affected by sleep changes and disturbances (103); for example, sleep is directly involved in the secretion of GH, and it is known that with increasing age there is a reduction in GH, in addition to the reduction in muscle mass. Age-related sleep problems potentially act intracellularly by inhibiting anabolic hormone cascades and enhancing catabolic pathways in skeletal muscle (103).

Age-related sleep changes and sleep disorders frequently observed at advanced ages reduce IGF-1 and testosterone secretion which potentially decrease IGF-1/PI3K/Akt and mTOR activity and increase myostatin expression, decreasing MPS and enhancing MPB (104). Furthermore, age-related increases in circadian cortisol levels can upregulate REDD1, which reduces mTOR pathway activity and decreases protein synthesis (104). Additionally, cortisol activates FOXO, enhancing atrogin-1 and MuRF-1 expression, which also promote muscle atrophy and ageing is associated with increased evening cortisol levels (103).

1.2.3 Definition and assessment of sarcopenia

1.2.3.1 Muscle Mass

The term muscle mass incorporates muscle quantity and muscle quality. Muscle quality is contributed by muscle size, fibre type, architecture, aerobic capacity, intermuscular adipose tissue, fibrosis and neuromuscular activation (139), therefore tools to measure mass can now be utilised to assess quality. In some cases, muscle quality can be defined as muscle function delivered per unit of muscle mass (59, 139) or the ratio of muscle strength to muscle mass or volume (140-142). There is yet to be a globally used quantitative definition to determine muscle quality. There are a number of assessment tools used to measure muscle mass, and function (in terms of strength and performance), in both clinical practice and research (Table 1.1).

Table 1-1: Summary of muscle mass assessment measures

Sarcopenia parameter	Clinical practice	Research methods
Muscle mass	Appendicular skeletal muscle mass (ASMM) by dual-energy X-ray absorptiometry (DXA)*	Mid-thigh muscle cross-sectional area by computerised tomography (CT) or MRI
	Whole body skeletal muscle mass (SMM) or ASMM predicted by bioelectrical impedance analysis (BIA)*	Muscle quality by mid-thigh or total body muscle quality by muscle biopsy, CT, MRI or magnetic resonance spectroscopy (MRS)
	Lumbar muscle cross sectional area by CT or MR	

Lumbar cross-sectional area (CT or MR)

This method of assessing muscle mass stems from repeated imaging from clinical research and practice at lumbar vertebral level 3 (L3). It is associated with high equipment cost and some radiation depending on the technique, is not portable and requires experienced and trained staff to use.

Femur cross sectional area (CT or MRI)

This is an evolving technique and is superior due to the reduced amount of radiation. It initially evolved from research studies and is a good predictor of whole-body skeletal muscle mass (59), with some data suggesting it is superior to lumbar L1-L5 muscle area (143).

Ultrasound quadriceps

Ultrasound is cheap, accessible and widely available. It has been validated in older adults with good intra and inter observer validity and is comparable to other methods of measuring muscle mass such as DXA and CT (59).

Dual-energy X-Ray Absorptiometry (DXA)

This mode of imaging is commonly available and non-invasive; however it does deliver a degree of radiation. It can determine muscle quantity, such as total body lean tissue mass or appendicular skeletal muscle mass, which can be adjusted for weight and BMI. However, it is influenced by the hydration of the patient and there are variabilities with the adjustment method (59).

BIA (Bioelectrical impedance analysis)

This technique provides an estimate of muscle mass based on whole-body electrical conductivity by using a conversion equation which has been calibrated with a reference to DXA-measured lean mass in a specific population. It is cost effective and portable; however the measurements are influenced by hydration status of the patient and there needs to be more validation in different ethnic cohorts and its reproducibility (144).

1.2.4 Muscle function

For the purpose of this thesis, I have combined the term function to include strength and performance. Table 1.2 summarises the tests below. Physical performance can be seen as an

objectively measured whole-body function related to locomotion, including balance and the nervous systems (145). It is not always possible to use certain physical performance measures in specific patient groups, if the performance is impaired by an external factor such as a gait disorder (59).

Table 1-2: Summary of muscle function tests

Muscle function parameter	Clinical practice	Research methods
Muscle strength	Grip strength	Isokinetic dynamometry
	Chair stand test (chair rise test) Chair stand test (5-times sit to stand)	
Muscle performance	Gait speed Short physical performance battery (SPPB) Timed-up-and-go test (TUG) 400-meter walk or long distance corridor walk (400-m walk or-6 minute walk test (6MWT)	

Hand grip strength

The most commonly used test to assess strength is hand grip strength (HGS). It is simple, inexpensive, easy to use and portable. A calibrated hand-held dynamometer is used. Low grip strength (<27kg for men, <16kg for women) is a validated, strong predictor of quality of life, poor patient outcomes and mortality (59, 60, 146).

Chair stands

This test, similar to HGS, is simple, inexpensive, easy to use, and portable. It measures the amount of time needed for a patient to rise five times from a seated position without using upper limb strength and can be used as a surrogate marker of quadriceps strength (59, 147). A cut off of > 15 seconds for five chair stands is defined as low strength (59).

Gait speed

Gait speed is a rapid, safe and very reliable test in the assessment of sarcopenia (148). The most frequently used test is the 4-metre walking speed test; a single cut-off speed ≤0.8 m/s is advised by EWGSOP2 as an indicator of severe sarcopenia (59). Gait speed can predict adverse outcomes related to sarcopenia such as mortality and falls (149).

Other tests to assess gait speed are:

Timed get and up and go test : This evaluates physical function; the participants are asked to rise from a standard chair, walk to a marker 3 m away, turn around, walk back and sit down again. In older hospitalised patients, it is a good predictor of sarcopenia (59, 150).

6-minute walk test: Participants are asked to walk for six minutes on a straight, flat surface in an enclosed corridor and are allowed two breaks within that time period (151).

400 m walk test: This test assesses walking ability and endurance. For this test, participants are asked to complete 20 laps of 20 m, each lap as fast as possible, and are allowed up to two rest stops during the test (152).

Short physical performance batter (SPPB)

This test includes the assessment of gait speed, balance and a chair stand which gives a maximum score of 12 points, and a score of ≤ 8 points indicates poor physical performance (60, 153).

Isokinetic dynamometry

This technique assesses isometric torque methods and can be used to measure lower limb strength through knee flexion and extension (154). It does provide reliable values of muscle strength measured using isokinetic dynamometers. However the machine required to carry out these tests is large and expensive; therefore, its wider application in clinical practice is limited and it is mostly used in research studies (155, 156).

Muscle biopsy

Muscle biopsies have been used in research studies for assessment of protein turnover and muscle quality. The most commonly used technique is a biopsy from the vastus lateralis on the anterior thigh, as it has been validated for safety. This invasive technique does have risks, and whilst frequently used in healthy young populations, it is used in other cohorts less frequently due to increased risk profiles. This method is described in more detail in chapter 2.

1.2.5 Secondary sarcopenia

Secondary sarcopenia refers to the reduction of muscle mass and function due to inactivity, disease and/or inadequate nutrition (157).

1.3 Chronic Liver Disease

It is clear that chronic liver disease (CLD) is a catabolic inflammatory state that both enhances and causes sarcopenia in patients (71). CLD is a progressive condition where there is destruction and regeneration of the liver parenchyma leading to fibrosis and cirrhosis. It encompasses a variety of liver pathology including early inflammation, up to and including end stage liver failure. Liver disease is the 4th commonest cause of death in Europe and 14th worldwide (158) and 12th in the US (159). It is an increasing cause of morbidity and mortality in the UK and in developing countries (160). Whilst the main aetiologies were traditionally alcohol related liver disease (ArLD) and Hepatitis C (HCV), the prevalence of NAFLD is ever increasing, with the cost of liver related hospital admissions is also increasing (159).

1.3.1 Definitions

There are several definitions used within the field of CLD. Liver cirrhosis is defined by a progressive hepatic fibrosis characterised by the destruction of the hepatic architecture and formation of regenerative nodules (158). End stage liver failure (ESLF) is a term used to describe advanced liver disease, liver failure and decompensation (161), whereas end stage liver disease (ESLD) encompasses advanced liver disease where there are irreversible complications (162).

1.3.2 Pathophysiology

Cirrhosis is a characterised by tissue fibrosis and transformation of the normal architecture into abnormal nodules (163). Key histological features include diffuse fibrosis, regenerative nodules, altered lobular architecture and intrahepatic shunts between afferent (portal vein and hepatic artery) and efferent (hepatic vein) liver vasculature (163, 164).

The spectrum of chronic liver diseases begins with chronic inflammation from the aetiology of disease, activation of hepatic stellate cells and fibroblasts with subsequent fibrogenesis, angiogenesis with vascular occlusion and distortion (165). Liver fibrosis occurs in response to chronic inflammation and parenchymal damage, leading to hepatic fibrogenesis. There are spatial patterns of deposition of a fibrillar extracellular matrix with the liver parenchyma, due to degradation and remodelling (163, 166). This is detectable via non-invasive and invasive investigations such as transient elastography and liver biopsy (167). Different types of cell types are involved depending on the location of damage and aetiology; in hepatocellular damage, hepatic stellate cells predominate, whereas damage involving or adjacent to portal tracts, involve predominately myofibroblasts and fibroblasts (166) (Figure 1.4).

As a consequence of inflammation and fibrosis, angiogenesis is the next step in the progression of CLD. There is increased expression of cytokines, metalloproteases and growth factors such as platelet-derived growth factor, TGF-β1, fibroblast growth factor and vascular endothelial growth factor (VEGF), which promote angiogenesis and fibrogenesis (163, 168). Liver tissue hypoxia results from remodelling, oxidative stress and production of ROS (166), which simultaneously occurs promoting neo-angiogenesis and fibrogenesis and further distorting the hepatic vasculature with ineffective hepatocyte perfusion.

Portal hypertension (PHT) is a consequence of cirrhosis and results from increased intrahepatic resistance and increased portal and hepatic arterial blood flow. There is a decreased vascular resistance in both the splanchnic and systemic circulation resulting in an increased splanchnic blood flow. When the hepatic venous portal gradient rises to 10-12mmHg, this broadly correlates to thresholds from compensated to decompensated liver disease (163).

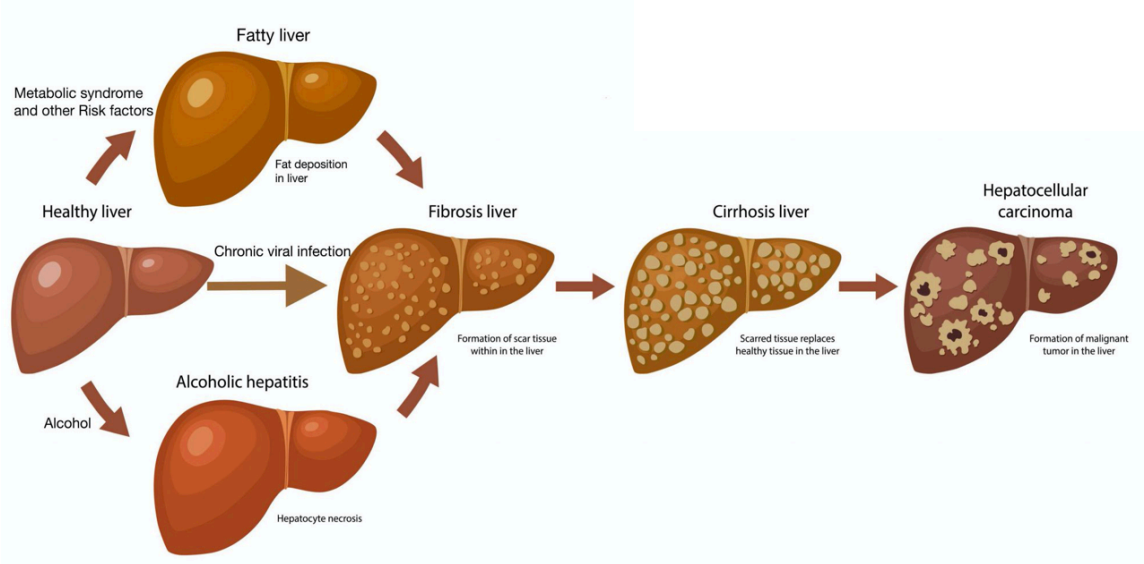


Figure 1-4: Progression of chronic liver disease

This figure demonstrates the development of cirrhosis and the progression through each stage of chronic liver disease. Created using Biorender.com

1.3.3 Aetiology

There are many different aetiologies of cirrhosis in the UK. The most common cause over the last decade is ArLD, with a sharp increase in NAFLD due to the epidemic of obesity and diabetes (169). Other aetiologies of liver disease include viral causes (HBV and HCV), metabolic causes such as haemochromatosis, Wilson's disease and autoimmune causes such as primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), autoimmune hepatitis (AIH) and seronegative hepatitis. Other non-common causes include cryptogenic cirrhosis, cardiac failure, and drug induced hepatitis (162). It is important to note that there may be more than one aetiology driving liver disease, for example some people with NAFLD may also consumed excessive amounts of alcohol that will also contribute to the ongoing inflammation in the liver.

1.3.4 Diagnosis

The diagnosis of chronic liver disease involves a clinical assessment and investigations. Patients may have features of CLD on clinical examination. Patients will undergo a number of standardised investigations to investigate the aetiology, assess severity and determine prognosis (162, 170) (Table 1.3). Liver cirrhosis does not require a liver biopsy to confirm the diagnosis, however it may be required in some patients.

Table 1-3: Routine investigations in diagnosing chronic liver disease

Blood work		Imaging	Histology
Liver screen	Immunology: Immunoglobulins IgA, IgM and IgG, AMA, ANA, ASMA, LKM Virology: HAV, HBV, HCV, CMV, EBV, HIV Metabolic Caeruloplasmin, iron studies, ferritin, HFE genotyping	Liver ultrasound with portal venous doppler	Liver biopsy (percutaneous or transjugular)
Liver biochemistry	AST, ALT, ALP, GGT	CT triple abdomen and pelvis	
Renal function	Sodium, Potassium, Urea, creatinine, EGFR	MR liver	
Full blood count	Hb, platelets, WCC, neutrophil count, MCV	Transient elastography	
Clotting profile	INR, PT, APTT	Portal venography	

IG (immunoglobulin), AMA (anti mitochondrial antibody), ANA (anti-nuclear antibody), ASMA (anti smooth muscle antibody), LKM (liver kidney antibody), HAV (hepatitis A virus), CMV (cytomegalovirus), EBV (Epstein barr virus), HIV (human immunodeficiency virus), HFE (hereditary haemochromatosis), AST (aspartate aminotransferase), ALT (alanine transaminase), ALP (alkaline phosphatase), GGT (gamma glutamyltransferase), eGFR (estimated glomerular filtration rate), Hb (Haemoglobulin), WCC (white cell count), MCV (mean corpuscular volume), INR (international normalised ratio), PT (prothrombin time), APTT (activated partial thromboplastin time).

1.3.5 Complications of cirrhosis

The complications of CLD fall into four main domains: 1) Portal hypertension which manifests with ascites, hypersplenism, and varices (oesophageal, gastric and rectal); 2) synthetic dysfunction with hypoalbuminaemia and coagulopathy; 3) hepatic encephalopathy; 4) hepatocellular carcinoma. Decompensated cirrhosis is defined as an acute deterioration in liver function in a patient with cirrhosis and is characterised by jaundice, ascites, hepatic encephalopathy, hepatorenal syndrome or variceal haemorrhage (171, 172). The most common precipitants of hepatic decompensation include infections, gastrointestinal (GI)

bleeding, high alcohol intake / alcohol-related hepatitis or drug-induced liver injury although no specific cause is found in approximately 50% of case (173).

Compensated cirrhosis can be asymptomatic or discovered incidentally. These patients do need to be identified and managed to reduce the risk of progressing to decompensated cirrhosis (174, 175). Compensated cirrhosis carries a significantly higher survival rate (159). Decompensation is an important development in those with liver disease, as a huge increase in mortality and morbidity occurs (160, 176), in addition to impact on their quality of life. Compensated liver cirrhosis has an annual mortality of 1-3.4% whereas decompensated cirrhosis has an annual mortality of 20 to up to 67% (160).

1.3.6 Prognosis

Prognostication is important for predicting progression of disease in those with early or asymptomatic cirrhosis, and to predict survival and the need for transplantation in those advanced disease. There are non-invasive biomarkers such as enhanced liver fibrosis (ELF) testing and Fibroscan which are being increasingly used to prognosticate in the community or early disease (162).

Historically, quantification of liver disease was first established by the Child Pugh (CP) scoring system (177), which is based on 3 biochemical values (serum bilirubin, albumin and INR) and 2 clinical measures (ascites and hepatic encephalopathy). It is scored from a minimum of 5 to a maximum of 15. There are three classes A (score of 5-6), B (score of 7-9) and C score of (10-15). Classes A, B and C represent a 1-year survival of 100%, 80% and 45% respectively (178, 179). Further robust scoring systems such as the Model of End stage Liver Disease (MELD) and

more recently UK Model of End stage Liver Disease (UKELD) score are frequently used. These scores represent the chronicity of liver disease. They are important as they allow prediction of mortality. The MELD score is calculated using INR, serum sodium, creatinine and bilirubin concentrations and it predicts 3-month mortality. It ranges between a score of 6 -40. A MELD score > 30 is associated with a 52.6% 90-day mortality (180-183). UKELD score is calculated using INR, serum sodium, creatinine and bilirubin concentrations and predicts 1-year mortality. A score of >60 is predictive of 50% 1- year mortality. It is used in the UK for transplant eligibility (184, 185).

1.3.7 Prevention and Management of CLD

There is an array of management strategies for CLD with the followings aims: to delay the progression of cirrhosis; prevent precipitants for decompensation; manage complications of cirrhosis. The only curative treatment for cirrhosis is liver transplantation.

1.3.8 Lifestyle measures

There are several lifestyle factors that should be address in the management of CLD. These include alcohol abstinence, optimisation od metabolic risk factors and smoking cessation as they can increase the risk of decompensation and progression of liver disease, and the development of HCC (165, 186). Furthermore, in HCV and NAFLD, alcohol is an independent risk factor for HCC (165, 187, 188) and increases the risk of HCC (189).

1.3.8.1 Aetiology specific treatments

The underlying cause of CLD needs to be addressed to stop the ongoing insult to the liver and to prevent disease progression. These include antiviral treatments for viral hepatitis,

immunosuppression for autoimmune aetiologies, venesection for haemochromatosis and copper chelation or zinc treatment for Wilson's disease.

1.3.8.2 HCC Surveillance

Each patient with cirrhosis should undertake an HCC surveillance screening in the form of a 6 monthly AFP blood test and US of the liver and portal system, as there is an increased risk of developing HCC with cirrhosis (190, 191).

1.3.8.3 Management of portal hypertension

Portal hypertension (PHT) is defined as a hepatic venous pressure gradient (HVPG) > 5mmHG. There is a risk of developing varices and ascites as a result. All patients with cirrhosis should undergo a screening upper GI endoscopy to asses for portal hypertensive gastropathy and varices (192, 193). There are prophylactic options including non-selective β blockers and endoscopic variceal band ligation (194). Ascites occurs as a consequence of PHT and insufficient water and salt homeostasis (195). This can be managed with dietary advice (no added salt) and diuretics such as spironolactone and furosemide (196, 197). For those that are non-responsive to diuretic treatment, a transhepatic portal systemic shunt can be considered in a select cohort (198). Alternatively, frequent large volume paracentesis can be performed to offload the additional fluid (195).

1.3.8.4 Management of complications and precipitants of decompensation

Hepatic encephalopathy has an associated 1-year mortality of up to 64% (165) and can occur due to precipitating events such as infection and be transient. For those in which it is long standing it is managed with lactulose as first line and rifaximin (non-absorbable antibiotic)

(172, 196). Any patients with HE are advised not to drive and to inform the DVLA (199). There are a number of precipitants for decompensation. These include infection, constipation, and electrolyte imbalance. (172, 196).

1.3.8.5 Liver transplantation

This remains the only curative treatment for CLD, cirrhosis and it's complications including HCC. The process of transplant assessment, eligibility listing, prioritisation and organ allocation is decided via national allocation since 2018 and National Health Service Blood and Transplant (NHSBT) guidance in the UK to ensure equity and universal scoring as there is a shortage of organ donation (200-202). Therefore, this option is only suitable for selected patients. Importantly, mortality of liver transplant waiting list patients is high, at approximately 20% being removed due to death or deterioration (203, 204).

1.4 Sarcopenia in Chronic Liver Disease

Cirrhosis is a catabolic state in which muscle protein breakdown exceeds synthesis resulting in sarcopenia (205). Sarcopenia is associated with higher rates of complications of cirrhosis (e.g. hepatic encephalopathy and infections), increased hospital admissions, length of admissions and premature mortality (206-209). In addition, it is associated with poorer clinical outcomes after liver transplantation (e.g. rejection, length of hospital stay and mortality), in addition to reduced quality of life and lack of functional independence (210, 211).

As liver transplantation remains the only cure for patients with decompensated cirrhosis, early recognition and management of sarcopenia is a critical aspect of the care of patients with cirrhosis. The prevalence of sarcopenia in cirrhosis ranges from 30–70%, depending on

the diagnostic tools utilised and the severity of the underlying liver disease (212). Increasing rates of computed tomography (CT)-defined sarcopenia are observed with worsening liver disease severity, as defined by the Child–Pugh score (CP A = 10%; B = 34%; C = 54%) (212).

Liver disease aetiology is important in sarcopenia, as there are liver disease-specific drivers that may potentiate the progression of sarcopenia; for example, alcohol promotes skeletal muscle protein breakdown, cholestasis promotes fat and vitamin D malabsorption in primary biliary diseases, whereas sarcopenia and NAFLD share similar pathological mediators, such as chronic inflammation, insulin resistance and physical inactivity (205, 213, 214). Challenges arise when the prevalence of sarcopenia may be masked by the coexistence of morbid obesity (termed 'sarcopenic obesity'), which is most notable in patients with NAFLD (215).

The definition of sarcopenia in CLD differs from the earlier described general age-related definition. Sarcopenia has been termed as loss of muscle mass, where frailty incorporates the contractile dysfunction and the disordered consequence that occurs (216-218). Most recently the North American Expert Opinion statement on sarcopenia have issued their rationale of using muscle mass alone to define sarcopenia, whilst incorporating muscle dysfunction in their definition and concept of 'frailty' (219). Sarcopenia (defined as loss of muscle mass alone) is associated with increased mortality, morbidity, physical disability and poor quality of life in CLD and post LT (205, 206). Interestingly, by including measures of muscle strength (HGS) and function, within the concept of frailty, these have been shown to be independent predictors of mortality in ESLD and after liver transplant, irrespective of the severity of the underlying liver disease (220, 221).

The extent of sarcopenia and the factors driving muscle loss in these conditions remain poorly defined, limiting strategic or personalised therapeutic intervention. Over the past decade, there has been increased recognition of the importance of sarcopenia in patients with liver disease, in particular those with ESLD and those undergoing liver transplantation (LT). The prevalence ranges from 20 to 70% in these populations, with higher rates in men (222, 223). This raises important questions in this cohort as to the pathogenesis of sarcopenia, its reversibility and what is the optimal therapeutic approach (205, 224, 225).

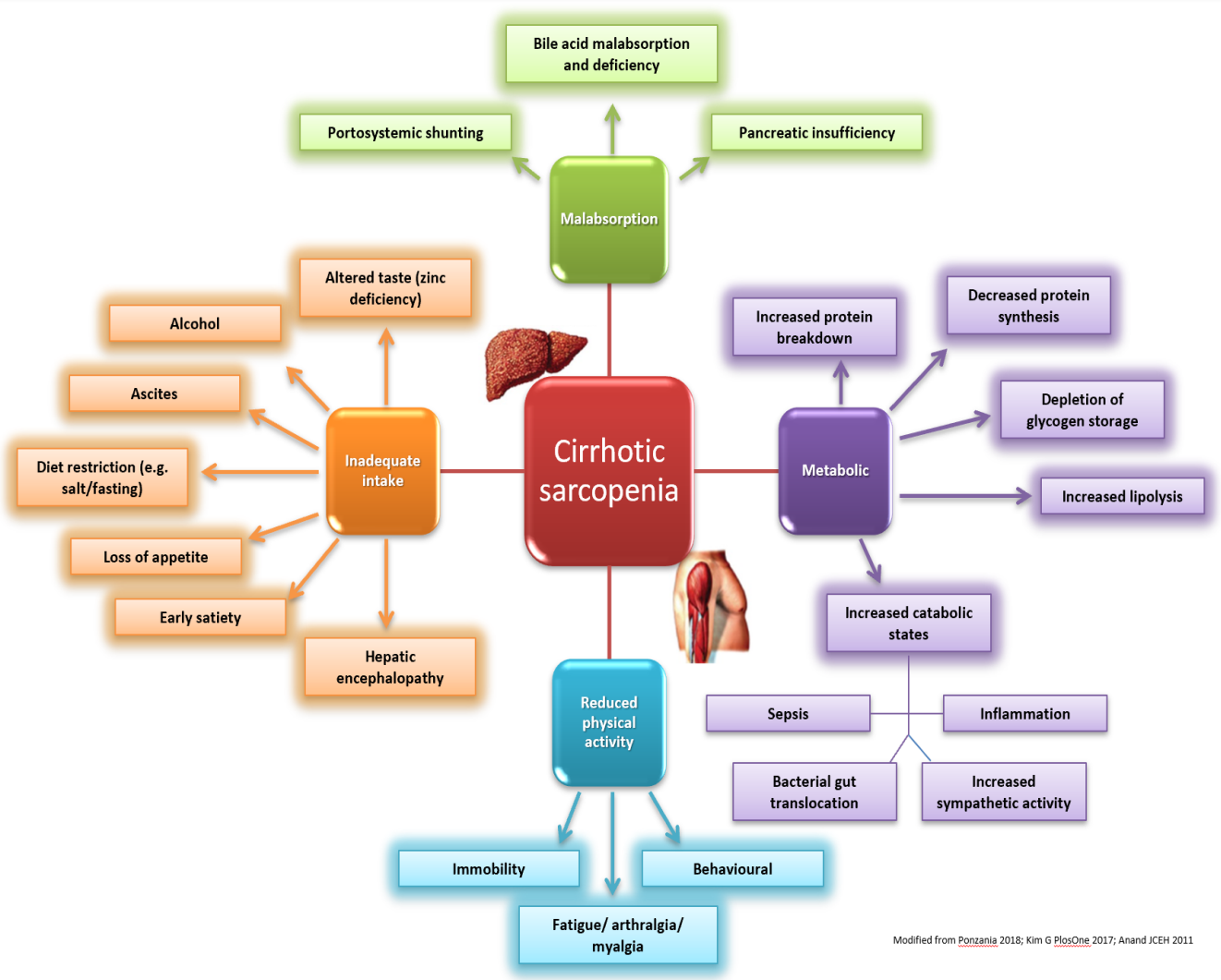


Figure 1-5: Aetiology of sarcopenia in chronic liver disease

This figure describes the main causes of sarcopenia in CLD; through inadequate intake, malabsorption, reduced physical activity and metabolic drivers. Reproduced from A. Dhaliwal et al, Frontline Gastroenterology (226).

The recognised causes for sarcopenia in ESLD are multi-factorial and largely revolve around a state of severe protein catabolism, poor nutritional intake, chronic inflammatory state and barriers to physical activity (fatigue (205), fluid retention (227), encephalopathy (197)) (Figure 1.5). However, with the exception of dietetic input and exercise advice, there is a paucity of established therapies. The only current curative treatment for ESLD remains LT, which is one of the single largest, surgical physiological stressors the human body encounters in modern day health care (170). Therefore, it remains paramount to increase our knowledge of the clinical assessment tools and therapeutic strategies surrounding sarcopenia in order to optimise the patient through the various stages of CLD.

The literature at present is still lacking evaluation of muscle mass in different ethnicities with liver disease (i.e. majority of data from North America and the Far East Asia) and rarely accounts for the gender disparity in sarcopenia (mortality: male>female) (228). Furthermore, the evolving prevalence of sarcopenia in parallel with the epidemic of obesity (leading to 'sarcopenic obesity') poses a significant challenge in the field of CLD, with rates of 20-40% reported in patients awaiting LT (229, 230). Patients with sarcopenic obesity have worse 1-year post-LT survival than those with non-sarcopenic obesity (54% vs 84%) in a living donor LT cohort (231). There remains a paucity of data in patients with a body mass index (BMI) >35-40 kg/m^2, in whom sarcopenia may go unrecognised using measures of muscle mass alone in the absence of functional/physical assessments. Research is required to counteract this.

1.5 Mechanisms of sarcopenia in CLD

There are several mechanisms currently known to drive sarcopenia in CLD (Figure 1.6), some of which differ from the those driving age-related sarcopenia, though the process is still not fully understood (Figure 1.6)

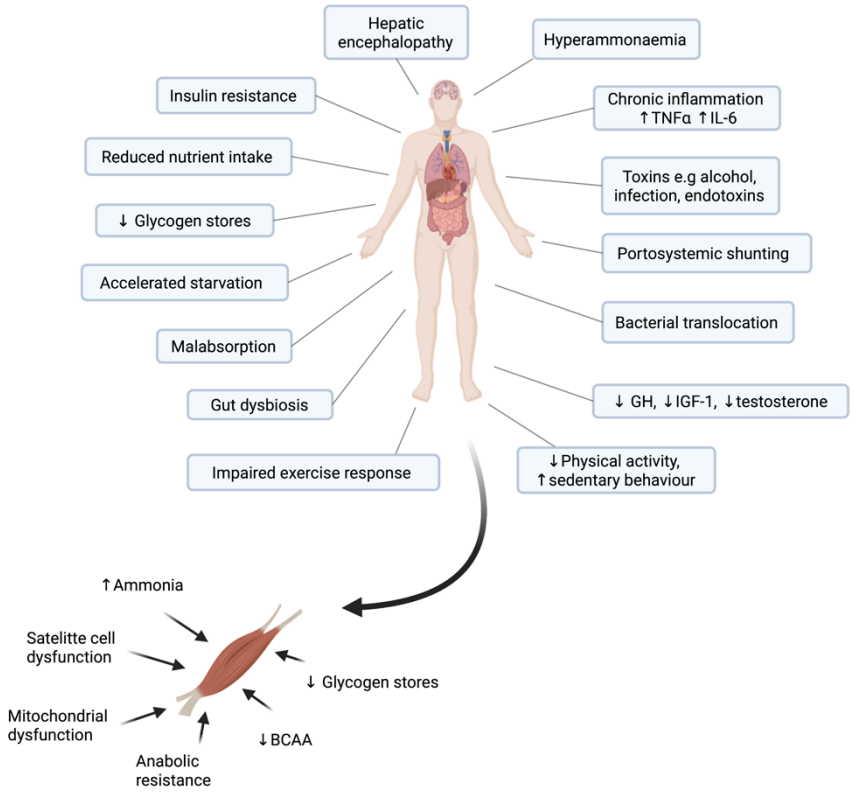


Figure 1-6: Overview of the mechanisms involved in sarcopenia in chronic liver disease

This figure demonstrates all the systemic factors that contribute to the development of sarcopenia in CLD, further highlighting the specific factors that dysregulation muscle metabolism and alter its' homeostasis. TNFα (tumour necrosis factor alpha),IL-6 (interleukin 6), GH (growth hormone), IGF-1 (insulin like growth factor 1), BCAA (branched chain amino acids). Created with Biorender.com.

1.5.1 Altered responses to anabolic stimuli

Muscle protein turnover and regeneration is regulated by three main processes: the anabolic mTOR signalling cascade; satellite cell responses; and the catabolic UPS (232). MPB in CLD is stimulated by inflammation, physical inactivity, increased myostatin, and mitochondrial

dysfunction as described below. Protein turnover in cirrhosis is dysregulated and likely reduced (205, 232, 233) and myostatin expression is increased in skeletal muscle in CLD (232, 234).

Other pathways involved in MPB are namely the UPS, lysosomal autophagy pathway and the calcium dependent calpain pathway. Perturbations of these processes in CLD has several causes upstream including hyperammonaemia (235, 236), endotoxaemia (205), altered hormones (232) and nutrients (207, 235, 237).

1.5.2 Accelerated starvation

1.5.2.1 Normal fed to fasted state metabolism

Skeletal muscle protein synthesis and proteolysis, is dependent on the metabolic state of the body e.g. post prandial, fed, post absorptive or fasted (238). Following ingestion of a meal and in post prandial states which last 4-6 hours post ingestion, MPS is stimulated by dietary substrates; glucose, fatty acids (FA), nitrogen, and amino acids (AA). AA are directly utilised by skeletal muscle. Raised glucose levels stimulate insulin, which increases glucose uptake in all cells, to reduce glucose concentration. Excess glucose is converted to glycogen for storage in muscle, adipose and the liver. MPB is supressed post prandially (137, 239, 240). Additionally, the liver absorbs AA post-prandially, which are converted in ketoacids. Conversion AA to ketoacids involves deamination which forms ammonia. Normally this will then be converted into urea by the liver and renally excreted (241).

In a post absorptive/ fasted state, non-essential protein synthesis is inhibited. Glycogen stores are relied upon for energy (137). During fasting, gluconeogenesis, lipolysis and proteolysis are

triggered for provide energy. Proteolysis in skeletal muscle is the primary source for AA (205) which are exported and utilised for essential protein synthesis e.g. enzymes, immunoglobulins. Energy utilisation is switched from glucose to FA, thus adipose is catabolised via lipolysis for FA production. Gluconeogenesis is still activated, despite this switch, as glucose is the preferred substrate for certain organs (242, 243).

Cirrhosis is a highly catabolic state (226); the duration of fasting when proteolysis and muscle loss occur is longer (244) and this post prandial fed state does not last the usual 4 -6 hours – it is 'accelerated' (205, 245). In cirrhosis, an overnight fast equates to 72 hours of starvation response (197, 246). Indirect calorimetry studies show a rapid switch from the carbohydrate and glucose substrate to FA and AA (for gluconeogenic) substrates, therefore there is an increase in fatty acid oxidation, gluconeogenesis and ketogenesis, driving this accelerated catabolism (247). This is a switch from high to low respiratory quotient (242, 248). Therefore, proteolysis occurs much faster in this hypermetabolic state to provide AA for gluconeogenesis. Skeletal muscle proteolysis produces both BCAA and aromatic amino acids. BCAA are catabolised in the muscle, whereas aromatic amino acids are primarily metabolised in the liver (205). Therefore in cirrhotic states, there is an lower concentration of BCAA available for in skeletal muscle for MPS and for ammonia clearance (249) and an increased level of aromatic amino acids, that cannot be utilised by skeletal muscle, due to the porto systemic shunting and hepatocellular dysfunction within the liver(205, 250).

In cirrhosis, the AA deficiency triggers an integrated cellular stress response to restore protein homeostasis whereby mTORC1 is inhibited and autophagy is promoted to preserve levels of AA. In hyperammonaemia, an adaptive integrated cellular stress response occurs to

counteract this, in the form of an increase in SLC7A5/LAT1 AA transporters (232, 251). This transporter increasing leucine uptake as an AA exchanger to generate ATP required for protein synthesis (252). Whilst further mechanistic specifics are currently unclear, this altered metabolism is associated with poor clinical outcomes (238, 253).

1.5.2.2 Inadequate nutrient intake

The imbalance of increased muscle protein catabolism and reduced protein synthesis occurs in patients with CLD especially ESLD due to inadequate oral intake and impaired nutrient intake. The daily intake of total calories, proteins and carbohydrates is inadequate in approximately 85–95% of patients referred for LT assessment (254), further reducing AA substrate availability for MPS. This reduced intake results from a variety of factors; early satiety pronounced in those with ascites, anorexia, nausea, dysgeusia, diet unpalatability, dietary restriction and hospital admissions. Furthermore, HE causes fluctuating consciousness and cognition thus less intake. (226, 255). This Impaired macronutrient and micronutrient intake results in significant deficiencies. Fat soluble vitamin deficiencies (Vitamin A, D, E, K) are common in those with cholestasis, and folate, thiamine deficiencies are frequent in those with ArLD (256). Vitamin D deficiency is prevalent in patients with cirrhosis with prevalence rates between 64-92% (257).

1.5.2.3 Malabsorption and impaired nutrient uptake

There are many causes for malabsorption in those with CLD. Cholestasis alters the enterohepatic circulation of bile salts. Biel salt are an integral part of fat absorption and metabolism; therefore, bile acid dysregulation can cause malabsorption of fatty acids and fat-soluble vitamin deficiencies. Pancreatic enzyme deficiency or insufficiency can also co-exist,

further worsening this (258). Malabsorption in the small bowel can lead to magnesium, zinc, ferritin, folate and B12 deficiencies. Portosystemic shunting can cause malabsorption as nutrients bypass the liver (259). Normally nutrients from the gut are absorbed into the liver, via portal system. By bypassing this or 'shunting,' substrate absorption is reduced (259).

Over colonisation of the small intestine and bacterial overgrowth impairs microvilli structure and absorptive function (258). This can be enhanced by bacterial translocation from conditions such as spontaneous bacterial peritonitis, further disrupting the intestinal flora (260). Dysbiosis has been observed in cirrhosis altering the normal floral balance, potentiating further enteropathy (260). Drug related malabsorption can occur, with commonly used drug treatments such as antibiotics, laxatives, and diuretics (258).

1.5.2.4 Protein turnover in CLD

Whole body turnover studies using labelled phenylalanine and leucine substrates, have produced differing results in CLD (205, 236, 237, 252, 261). Studies measuring MPS use a primed constant infusion of tracers to label muscle proteins and serial muscle biopsies, following a single dose of BCAA. This has shown similar rates of MPS in cirrhotic and controls however this is may be due to differences in the fasted state rather than the fed state (252).

Direct measures of MPB are challenging in tracer studies. There are studies using increases in 3 methyl histidine (3MH), which is formed by the methylation of peptide bound histidine in actin and myosin. Following myofibrillar catabolism, 3MH cannot be utilised and is excreted in urine (233, 238). Whilst some studies observing arteriovenous difference and 3MH release measurements have shown impaired skeletal MPS and increased MPB (233), the results of these studies remain conflicting.

1.5.2.5 Alterations to substrate availability

Whole body substrate utilisation studies using indirect calorimetry show accelerated starvation in CLD because fatty oxidation and gluconeogenesis are increased in the early post absorptive and fasting state as described above (239, 242). Amino acids are used as a second line substrate for gluconeogenesis as fatty acids cannot be used and glucose is the preferred substrate. The primary source of BCAA is proteolysis from skeletal muscle which is already reduced in cirrhosis (262). Additionally, the phenomenon of anabolic resistance, may also occur in those patients with cirrhosis, suggesting a blunted response to increased amino acid availability or ingestion (239).

1.5.3 Liver muscle axis - hyperammonaemia

Hyperammonaemia is observed in CLD. Ammonia is generated by AA metabolism, purine metabolism and enterocyte glutamine activity and urealysis in the gut (219, 236, 237). The liver usually catabolises ammonia and detoxifies it. There are increased levels of ammonia in CLD due to hepatocellular dysfunction, portocaval/systemic shunting and impaired ureagenesis (238). The direct mechanism of this remains unclear at present. In liver cirrhosis, there is increased muscle uptake of ammonia, as skeletal muscle is a metabolic partner to the liver as evidenced in other studies, and attempts to compensate in ammonia clearance in hyperammonaemic states (263). This compensatory mechanism may be as a neuroprotective response (263).

Ammonia is usually formed from an anapleurotic pathway whereby glutamine and glutamate are converted into ammonia and alpha ketoglutarate (αKG), catalysed via glutamate dehydrogenase, which are then utilised in the tricarboxylic acid cycle (TCA) for generation of

ATP. In hyperammonaemic states such as cirrhosis, the existing increased levels of ammonia, favour the pathway in an opposite catapleurotic manner as it inhibits αKG dehydrogenase, which catabolises αKG into succinyl Co-enzyme A, required for TCA cycle metabolism therefore resulting in less TCA metabolites. Additionally, ammonia also inhibits pyruvate dehydrogenase, which catalyses pyruvate into Acetyl Coenzyme A, another component required in the TCA cycle. This leads to impaired mitochondrial function and decreased levels of ATP synthesis, thus providing less energy for protein synthesis (236, 237, 251, 264). Reduced cellular ATP and increase in AMP stimulates activation of AMPK and impaired MTORC1 signalling, stimulating muscle proteolysis (MPB) (252) and a reduced rate of protein synthesis (MPS) (237, 265, 266) (Figure 1.3).

Hyperammonaemia further dysregulates protein synthesis due to an increase in myostatin expression and activation of NFκβ pathway (237, 267) (Figure 1.7). This elevated myostatin expression inhibits mTORC1 pathway, which regulates MPS, via impaired Akt signalling. This promotes proteolysis, and increases autophagy. Ammonia may potentially cause direct mitochondrial dysfunction and increase in ROS, which can overwhelm the adaptive response and cause a further increase in autophagy (205, 268, 269). It has been proposed that hyperammonaemia results in a greater rate of autophagy which drives the majority of the MPB, rather than UPS activation (232). To dispose of ammonia in skeletal muscle when there is a deficiency of ATP, there is a diversion of oxidative metabolism of pyruvate with redirection to lactate. This explains the increase of lactate in cirrhosis (238, 270, 271).

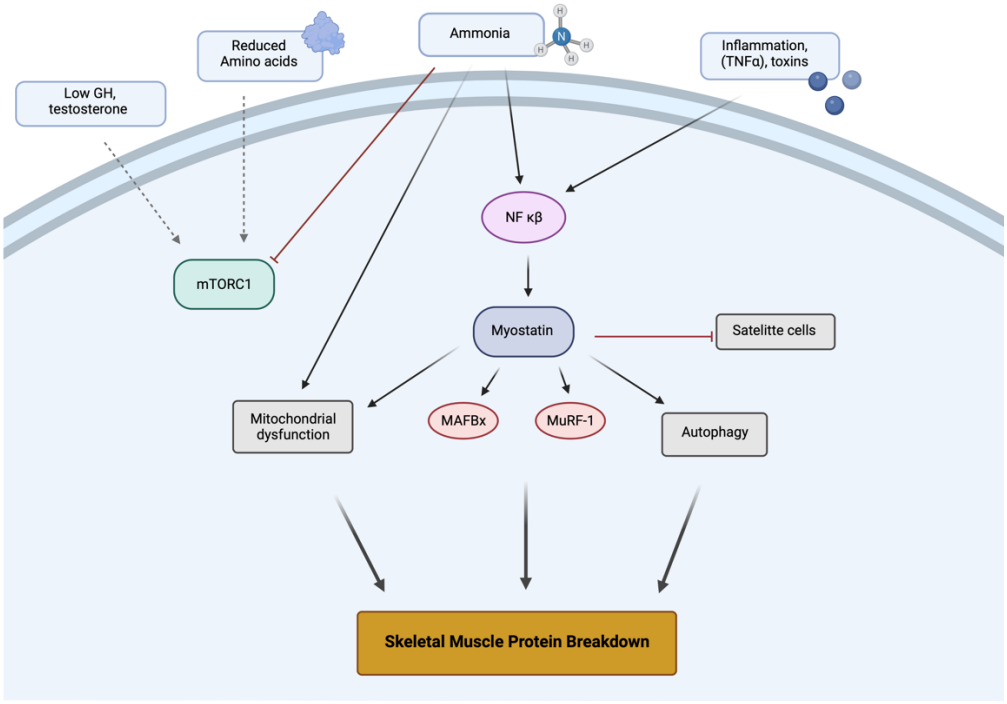


Figure 1-7: Regulation of muscle protein breakdown in chronic liver disease

Ammonia and inflammation stimulate NF-κβ pathway and myostatin which stimulate atrogene formation and muscle protein breakdown (MPB). It also stimulates autophagy and mitochondrial dysfunction to enhance MPB. Additionally, the lack of stimulation of mTORC1 from low levels of GH, testosterone and amino acids, means less protein synthesis for recovery. NK-κβ (nuclear factor kappa-light-chain-enhancer of activated B cells), GH (growth hormone), TNFα (tumour necrosis factor alpha), mTORC1 (mammalian target of rapamycin), MAFBx (muscle atrophy F box or atrogin-1) and MurF-1(muscle ring finger 1). Created with Biorender.com.

In sarcopenia where there is pre-existing low muscle mass, there is an added reduction in non-hepatic disposal of ammonia. As expected, HE is more frequent in sarcopenic cirrhosis than non-sarcopenic cirrhosis (272).

1.5.4 Contractile dysfunction

Fatigue and frailty are terms which describe the aspect of skeletal muscle contractile dysfunction which can manifest as reduced maximum contraction, impaired ability to sustain maximum contraction or the inability to perform repetitive activity (209, 217, 273-277). In

sarcopenia, there is a loss of contractile proteins, and this can be due to impaired mitochondrial function, reduced ATP generation and impaired contractile proteins (251). The increased oxygen consumption and lactate concentration combine to impair contractile function (238, 271).

Dysoxia is defines as impaired oxygen utilisation despite adequate delivery (270). A similar process occurs in hyperammonaemia and the subsequent impaired mitochondrial function, is a potential mechanism for contractile dysfunction in cirrhosis resulting in higher oxygen utilisation which results in fatigue and frailty (238, 251, 271). A reduced level of ATP also explains the reduced level of contractile force and function (278). Skeletal muscle hyperammonaemia impairs electron transport chain components, leading to increased NADH accumulation and electron leakage (251, 279).

1.5.5 Endocrine influences

Some of the main hormonal influences on sarcopenia in liver disease are described below.

1.5.5.1 Insulin resistance

Insulin resistance leads to high levels of circulating insulin which are not transduced by the insulin or IGF-1 receptors. Several factors drive this reduced signalling including inflammation and increased FA. It leads to impaired gluconeogenesis, reduced glycogen synthesis, increased free fatty acid uptake, increased lipolysis and altered triglyceride transport; all of which exacerbate proteolysis (280, 281). Increased free FA inhibits GH expression, which reduces the inhibition of myostatin and promotes MPB (205). Insulin resistance also results in reduced PI3K/Akt mTOR stimulation, reducing MPS(280). Obesity leads to insulin resistance

and myosteatosis which directly affects muscle fibre atrophy and contributes to sarcopenia in NAFLD (215).

1.5.5.2 Testosterone

Due to the changes to the HPG axis in men in cirrhosis, there is a reduction in testosterone (282, 283). This is due to increased aromatisation (which converts testosterone into oestradiol) and impairs 'beneficial' responses (238, 284, 285). Reduced levels of testosterone in men are seen with ageing and contribute to reduced muscle mass (286). Murine studies show that inhibiting aromatase increases testosterone levels and body weight (283, 287). Testosterone supplementation has been shown to result in some improvement in muscle mass in males with cirrhosis (283). The mechanism behind this is due to decreased myostatin expression, with subsequent increased MPS (288, 289). There is some debate as to long term safety with this supplementation and further studies are needed.

1.5.5.3 Growth Hormone

GH resistance and low levels of GH are reported in cirrhosis. GH activates local IGF-1 which inhibits myostatin and inhibits MPB, therefore loss of GH leads to reduced MPS and increased MPB (290, 291).

1.5.6 Inflammation

Cirrhosis itself is a chronic inflammatory condition. Proinflammatory cytokines such as TNFα, IL-1 and IL-6 are associated with increased MPB. TNFα stimulates activation of NF$\kappa\beta$ pathway which leads to activation of atrogenes (MuRF1 and atrogin 1), promoting proteolysis (281).

IL-6 activate myogenesis by stimulating the proliferation of stem cells, but also contributes to muscle atrophy as demonstrated in in-vivo animal models (292).

In CLD there is a low level of chronic inflammation in the form of low-level sepsis, resulting in circulating endotoxaemia. Endotoxins reduce translational efficiency via TLR 4 dependent signalling, which activates NFKB and the inflammatory responses which reduces MPS and activate autophagy(244, 293). This all leads to dysregulated proteolysis and muscle loss (294-296). Endotoxins impair the gut barrier function and can affect TNF dependent and independent pathways and potentially alter the gut microbiome. Increased gut permeability and impaired hepatic clearance of endotoxin leads to further muscle atrophy (205).

1.5.7 Alcohol

Alcohol excess can enable a greater degree of MPB than MPS, by increasing autophagy of skeletal muscle. Ethanol activates myostatin and can directly inhibit MTORC activity, therefore reducing MPS and promoting MPB (297, 298). Some researchers hypothesise that there is a synergistic effect of ethanol and the hyperammonaemic state of CLD, that enhance the degree of sarcopenia (297). Low levels of UPS activation are observed in patients with ArLD, again supporting the hypothesis that autophagy is the main driver for MPB. Alcohol may also generate ROS, with the existing mechanisms impairing mitochondrial function, leading to further autophagy.

1.5.8 Mitochondrial dysfunction

In addition to the reduction in the number of mitochondria that is observed in both CLD and ageing, there is evidence supporting impaired mitochondrial function in cirrhosis (299). With

reduced and impaired mitochondria, the rate of ATP required for MPS in skeletal muscle is reduced, resulting in a lowered rate MPS. In addition, the presence of myosteatosis can potentially lead to mitochondrial dysfunction, age related differentiation of muscle stem cells, and impaired lipid metabolism (300).

1.5.9 NAFLD

NAFLD is associated with sarcopenia due to reduced physical activity, insulin resistance and chronic inflammation (215, 301). Insulin resistance stimulates lipolysis and free FA are released in to the liver. Free FA inhibit IGF-1 signalling, which inhibits the PI3K/ Akt pathway and reduce the rate of MPS (302). As a result of IGF-1 inhibition, there is an increased production of atrogenes via the FOXO pathway and UPS (303). Furthermore, hyperinsulinaemia in hepatocytes triggers reduced gluconeogenesis and increased lipolysis and β oxidation inhibition (301). Therefore, lipids accumulate in the liver and muscle (myosteatosis) (215, 304, 305).

Obesity causes chronic inflammation as a result of the excess fat deposition in the viscera including the liver (306) and it is positively associated with insulin resistance (307). TNFα is the key cytokine driving this insulin resistance by inhibiting signalling through the insulin receptor. TNFα also increases UPS activity and in particular Atrogin-1 expression and reduces myogenesis (308, 309). Adipokines, cytokines secreted by adipose, also contribute to this inflammation (309).

1.5.10 Physical inactivity

It is well known that inactivity is a key driver of sarcopenia. It is well described that patients with cirrhosis and ESLD have reduced physical function and decreased levels of activity (256). A small study of 53 LT candidates with ESLD found 76% sedentary time in waking hours with a mean of 3000 daily steps.; with a significantly higher mortality in those who were physical inactive (310). It is also observed in compensated cirrhosis; a study in a cohort of compensated viral liver cirrhosis also demonstrated that those with sarcopenia had a lower number of daily steps, measured by pedometry, and that daily number of steps were independent risk factors for sarcopenia (311). More recently, a study by Golabi found that sarcopenia was related to physical inactivity (<150 minutes/week), amongst both NAFLD and non NAFLD groups (312). Furthermore, exercise capacity is reduced in cirrhosis (313). This may be due to both direct factors such as reduced ventilatory capacity, decreased inspiratory pressure and impaired VO_{2max} (the maximum rate of oxygen consumption measured during exercise, or maximal aerobic capacity) or indirect factors such as reduced oxygen delivery (314). This has been shown to have a negative impact on mortality.

VO_{2max} has been found to inversely correlate with MELD scoring, suggesting that a lower VO_{2max} is associated with a worsening severity of CLD. Additionally, Peak VO_{2max} was independently associated with survival in a study using CPET testing on LT candidates (315). A 6 min walk distance (6MWD) of less than 250 m was associated with a higher mortality risk in a cohort of ESLD patients awaiting LT, each 100-m increase in the 6MWD was significantly associated with increased survival (316). This emphasises the negative consequence of physical inactivity and poor exercise status in ESLD.

1.6 Assessment of sarcopenia in CLD

The tools used to assess sarcopenia in CLD are described below and summarised in Figure 1.9.

1.6.1 Nutritional assessments

Nutritional assessment may provide the first opportunity to identify malnutrition and those at risk of sarcopenia. There are several ways to perform a nutritional assessment including use of screening tools, food diaries and clinical assessment (Figure 1.9). All patients with liver cirrhosis, irrespective of BMI, should be screened and assessed for malnutrition. This can be performed using validated screening tools, anthropometry, functional assessment; and detailed food and diet history (71). In addition, clinical assessment in the form of functionality is important; observations on a patient's gait, rising from a chair, handshake, walking aids adds to this assessment (71).

1.6.1.1 Screening tools

Most screening tools have been developed for use in general hospital populations, However these don't take in account the specific risks and the alterations in body composition exhibited in CLD, for example, factors such as a raised body weight secondary to ascites or excessive fluid imbalance. If using standard screening tools, then it is imperative to adjust for those with CLD including altering frequency of screening and consideration of additional non-screened factors. The European Society of Enteral and Parenteral Nutrition (ESPEN) recommend the Nutrition Risk Screening (NRS) and the Malnutrition Universal Screening Tool (MUST) to screen hospitalised patients for malnutrition however these are not liver disease specific (317). The European Association for the Study of Liver disease (EASL) recommend the

Royal Free Hospital – Nutritional Prioritising Tool (RFH-NPT) based on the lack of liver specific screening tools.

There have been a few screening tools developed to incorporate the challenges in nutrition screening in those with CLD including the Subjective Global Assessment (SGA) RFH-NPT and Liver Disease Undernutrition Screening Tool (LDUST). SGA uses dietary habits of patients, weight loss in previous 6 months and information regarding gastrointestinal symptoms to assess malnutrition. Weight change is evaluated by proportionate and absolute loss (kg). Evaluation criteria of malnutrition measured by weight change are as follows: “small loss” stands for weight loss of <5%; “potentially significant loss” for weight loss from 5% to 10%; and “definitely significant loss” for weight loss of >10%. SGA classes are categorized into A, B, and C, where A is judged as “well nourished,” B “moderately (or suspected to be) malnourished,” and C “severely malnourished.” SGA is advantageous because it enables doctors or trained healthcare professionals to suggest clear treatment plans suitable for patients by examining them in various aspects and studies show that it is a simple and cost effective method compared to other assessment options (318). Liver specific SGA is insensitive for detecting muscle wasting compared to cross-sectional psoas muscle area in LT assessment in patients with raised BMI (319).

The RFH-NPT was developed to screen malnutrition in those with cirrhosis. It uses a short algorithm similar to the MUST but is adapted to cirrhosis. It does, however, include BMI. It independently predicted transplant free survival (320, 321), however it does need further validation in prognosis(71). The LDUST was developed in the United States. It was developed

to detect malnutrition in those with CLD for greater than 3 months. Whilst it is a quick test, there is no correlation at present with survival, prognosis or complications (322).

1.6.1.2 Food diaries

Food diaries can provide insight into dietary intake and help tailor nutritional optimisation for a patient with CLD. 24-hour diet intake recall is quick and can be specifically adapted to the individual. However, it is reliant on the patient's ability to do so. Alternatively, a 3-day to 7-day food diary provides a more detailed overview of typical intake (71, 226).

1.6.1.3 BMI

BMI alone can be used in initial screening for malnutrition; however, careful adjustments are needed in patients with fluid retention. A dry body weight and BMI should be recorded, using weight following paracentesis or via estimation by subtracting a percentage of body weight based on ascites (mild 5%: moderate 10%; and severe 15%) and peripheral oedema (mild 5%, moderate 10%) (197, 212, 222). Due to the increasing prevalence of sarcopenic obesity in patients with cirrhosis (197, 229), undernutrition (especially protein intake) should be formally assessed in all, irrespective of BMI. For example, in a 80 kg patient with mild ascites, estimated dry weight would be 80 kg −(5% of 80=4 kg)=76 kg (71).

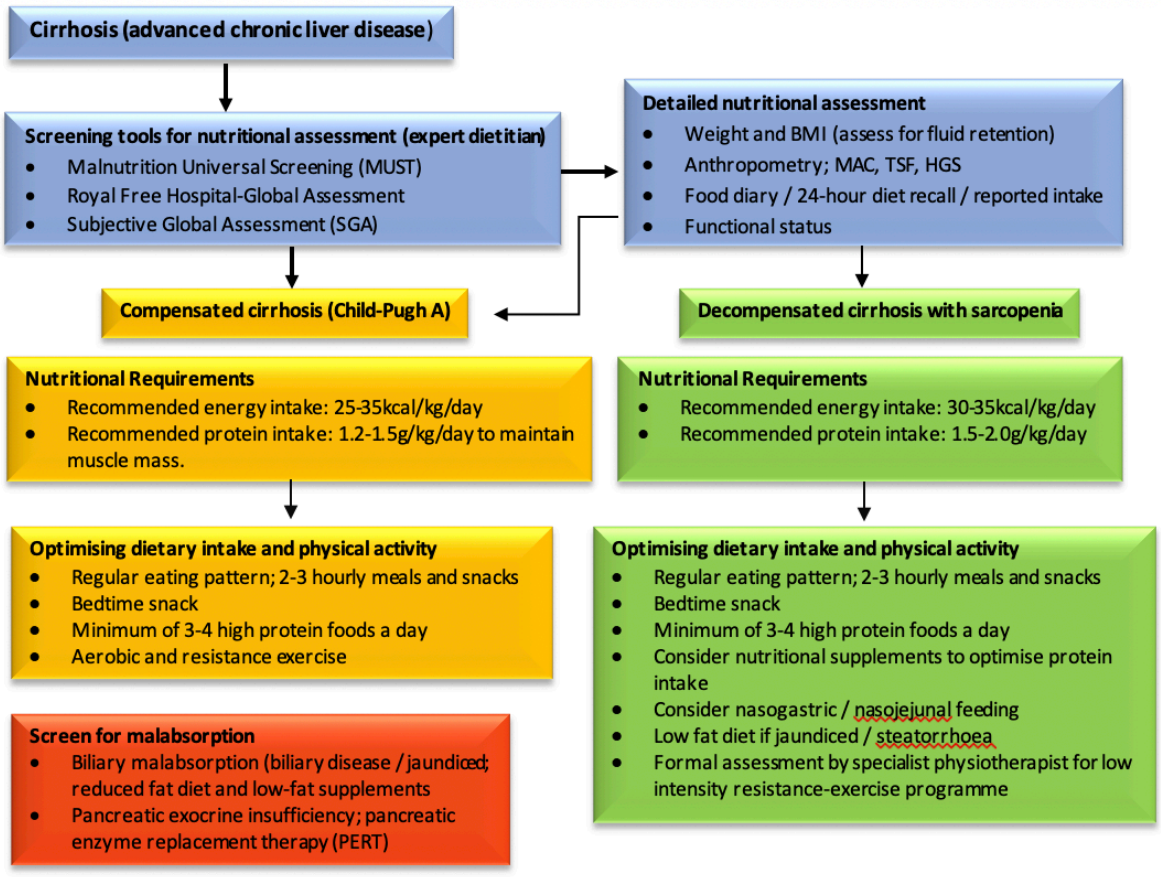


Figure 1-8: Overview of nutritional screening and optimisation for patients with chronic liver disease

This figure shows a flow chart highlights a pathway of nutritional assessment for those with CLD and provides an overview of nutritional management. MUST (malnutrition universal screening tool), SGA (subjective global assessment), MAC (mid arm muscle circumference), TSF (tricep skin fold thickness), HGS (handgrip strength).

1.6.2 Measuring muscle mass

1.6.2.1 Cross sectional imaging

CT

The most frequently used measure of muscle mass in patients with CLD, including pre and post LT, is skeletal muscle index (SMI) of the L3 lumbar level on CT. This is largely due to the increasingly routine use of abdominal CT imaging as part of the LT assessment and for the investigation of post-LT complications (323). In 2016, a large meta-analysis (19 studies),

consisting of 3802 patients awaiting or undergoing LT, investigated the utility of CT assessed skeletal muscle as a predictor of LT morbidity and mortality. Despite recognition of the fact that there was significant study heterogeneity (sample size, imaging, muscle type, liver disease severity), overall sarcopenia was associated with both pre-LT (HR 1.7) and post-LT (HR 1.8) mortality and to a lesser extent complication, including infection (324).

Due to a lack of standardised definition of sarcopenia and specific sex-defined cut-offs in these studies, widespread application to routine clinic practice has been challenging. However, the North American FLEXIT (Fitness, Life Enhancement, and Exercise in Liver Transplantation) Consortium has produced validated cut-offs for SMI at L3 to define sarcopenia in ESLD; namely < 50 cm2/m2 in men and < 39 cm2/m2 in women (219, 325). Sex-specific cut-offs of SMI correlated with LT waiting list mortality (218, 326), but the same was not true for psoas muscle evaluation (in the form of psoas muscle index (PSI)), which particularly underestimated clinical impact in males (325).

It is important to recognise the setting when applying SMI. For example, in patients with high Modified end-stage liver disease (MELD) scores and with acute deterioration, low SMI resulted in 4-fold increased risk of post-LT mortality in men (SMI <48 cm^2/m^2), but in contrast no differences were seen in women (327). Furthermore, a large cohort of 669 patients with cirrhosis highlighted that adding SMI onto the MELD (termed 'MELD-sarcopenia') improved the predictive accuracy of mortality, especially in those with a low MELD under 20 (211).

MRI

Guidance by the North American consortium suggests that SMI L3 of MRI produces comparable results to CT (218, 328). A study of 61 patients (329), with significant intraclass coefficient of 0.98 suggested no variance between CT and MRI. Additionally there were similar results for both sexes with a slight difference in mean muscle mass of 1.1g (95% CI 0.03-4.4) (329). Another study evaluated free fat muscle area at psoas level MRI imaging, which correlated to TPMT (transverse psoas muscle thickness) on CT, and that FFMA was an independent predictor of survival in this cohort of patients with decompensated cirrhosis after TIPSS (328). A recent retrospective study from 2020 evaluating TPMT on MRI images of 265 patients demonstrated excellent inter observer agreement for TPMT measurements (K+0.98 95% CI 096-0.98) (330). Whilst this is promising, further studies to confirm this are required. There are no studies to date to evaluate MRI quadriceps muscle mass in liver disease. There are established protocols and use of MRI for quadricep muscle imaging in other remits such as exercise (331, 332) , it remains unresearched in CLD.

Ultrasonography

There are only three studies to date which have investigated the use of US as a form of imaging (222, 333, 334). Two of these studies showed that the iliopsoas muscle group was accurately identifiable in the majority (80-+100%) of their cohort, with acceptable intra and inter operator variability (which is one of the main limitations with US generally), good correlation to CT and associated with prognostic markers (risk of hospitalisation) and mortality (333, 334). It is significantly limited by increased abdominal girth which is a finding in those with ascites and sarcopenic obesity (333-335).

Tandon et al evaluated quadricep muscle thickness (selecting rectus femoris and vastus intermedius at a specified point from the iliac crest and patella using a feather index method) in 159 patients with compensated cirrhosis. This method of quadricep muscle mass measurement reported excellent interobserver reliability (correlation =0.97) and was strongly associated with sarcopenia in both male and female patients with cirrhosis (AUROC 0.78 and 0.89 respectively) (222). It is an emerging, novel method of muscle mass assessment, which is significantlty less affected by variables such as ascites. Further studies are required. However with the increasing use of CT and MRI in routine clinical care and less limitations, it remains less utilised method of abdominal imaging.

1.6.2.2 Mid-arm muscle circumference

Anthropometry and more specifically mid-arm muscle circumference (MAMC) is cheap and can be safely used in out-patients to assess repeated measures of muscle mass. When performed by trained individuals, MAMC has good intra-/inter-observer agreement (336) and has been shown to predict mortality in patients with cirrhosis and those after LT (337). Surprisingly, some studies have shown that MAMC poorly correlates with CT and MRI. In addition to MAMC, targeting specific muscle groups (i.e. quadriceps, and in particular vastus lateralis) with ultrasound have shown promise in the elderly population (219, 338) and in isolated LT studies (222).

1.6.2.3 DXA and BIA

DXA and BIA are cheaper, safe, reproducible, time efficient and offer less radiation exposure compared to CT (339). However, their use is restricted in individuals with fluid retention (i.e. ascites, anasarca, hydrothorax) due to their inability to distinguish water from muscle and in

limited studies they have not been shown to be as accurate as CT imaging (340). ASMI has been used to overcome these limitations however their correlation to CT SMI is weak (341). A recent study in 429 men with cirrhosis demonstrated a strong association of upper limb ASMI with mortality (HR =0.27, P=0.004) (342). Whilst further studies are required to validate this, the use of DXA remains limited, however targeted use of upper limb ASMI may be validated in the future (335).

1.6.3 Measuring muscle function (strength and performance)

Various simple, cheap, non-invasive assessments of muscle function and physical 'functional' ability exist (Figure 1.9).

1.6.3.1 Frailty indices

To date, the main physical 'functional' performance measures that have been described in patients with CLD, include: Karnofsky Performance Status (KPS) (222, 343) and the Activities of Daily Living (ADL) scale (344, 345). There are other more recently used frailty indices including the Fried Frailty Index, the clinical Frailty scale and the Liver Frailty index (LFI) (335). For the purposes of this thesis, I will only further describe the LFI.

Liver Frailty Index

The LFI uses three performance measures; HGS, time to complete 5 chair stands, time held balance positions (side by side, semi tandem and tandem stance) to assess physical frailty in patients with ESLD (274). The LFI is a reliable test (correlation coefficient = 0.93) and is well-validated in cirrhosis (346).

1.6.3.2 Performance testing

There are several performance tests available in CLD; the commonly used tests include Short physical performance battery (SPPB) (310), 6-minute walk test (6MWT, also referred to as 6MWD[distance]) (347, 348), gait speed (349) and cardiopulmonary exercise test (CPET) (350, 351).

SPPB

The SPPB, which consists of chair stands, gait speed and balance (each scored out of 4; total 12), is associated with overall mortality in an LT cohort, with a cut-off < 10 increasing the odds of mortality by 2.5 (352). It is, however, limited in patients with higher levels of baseline physical function (SPBB 12 out of 12), as it has a ceiling score of 12, which means interventions may be overlooked in these individuals.

6 MWT

The 6MWT does not a have a ceiling effect, as it is a continuous scale of distance based on a sub-maximal exercise test used to assess aerobic capacity. In the last 5 years, cut-offs of distance have been described in relation to all-cause mortality (347, 348). The largest study to date of 213 out-patients with ESLD awaiting LT highlighted that a distance of <250 metres resulted in a 2-fold increased risk of mortality (348).

CPET

CPET requires specialist expertise and equipment (cyclometer or treadmill) (353). It is used in the context of those with ESLD undergoing assessment for LT. In 2016, Ney and colleagues performed a systematic review of 7 studies consisting of over 1000 patients and highlighted

that CPET variables (most notably peak VO_2 and anaerobic threshold (AT)) were independent predictors of pre-LT and post-LT mortality (350). The majority of these studies are retrospective in design and are limited by selection-bias, in that those with a low aerobic exercise capacity may have been declined listing for LT and thus their outcomes remain unknown. It is also important to highlight that no single cut-off for AT or VO_2 peak should be used in isolation to determine a patient's physical fitness for LT or in CLD. If CPET is utilised it should be taken in context with the patients ADLs, disease severity, co-morbidities and other markers of sarcopenia.

Of note, the Duke Activity Status Index (DASI) questionnaire, which takes less than 5 minutes to complete, has been shown to be a better predictor of death or major cardiac events than CPET in patients undergoing major non-cardiac surgery (354).

1.6.3.3 Hand grip strength

HGS, usually of the non-dominant hand, is the most readily used assessment of muscle strength and has the strongest evidence base in ESLD. Subsequently, HGS is currently recommended by both recent international guidelines European Association of Study of Liver (EASL) and European Society for Clinical Nutrition and Metabolism (ESPEN) in the assessment of all patients with cirrhosis and liver failure (197, 227). HGS has been shown to be a better predictor of adverse clinical outcomes than measures of muscle mass (355) and MELD (225). it can represent a quick, bedside global assessment of muscle strength in patients with ESLD as it correlates strongly with lower extremity muscle power (356). In a recent North American study of 145 male patients undergoing LT assessment, an increase in 1 kg of HGS was associated with a 6% reduction in mortality, independent of the baseline MELD (225). In

combination with MELD, HGS was significantly superior to MELD and CT muscle mass in predicting transplant-free survival on the LT waiting list (225). This however would need to be reproduced in a female population.

1.6.3.4 Accelerometry

Accelerometry is a method used to evaluate physical activity objectively, via wearing a wrist or body accelerometer. It has been suggested that those with higher volumes of regular moderate to vigorous PA are protected against mortality (335, 357, 358). Patients with ESLD awaiting LT, have amongst the lowest PA levels compared with healthy adults (359). Furthermore, a recent prospective study of 96,688 patients has shown that greater physical activity (additional 2500 steps) is associated with a 38% reduction in CLD and 89% risk reduction (HR 0.11 [0.02-0.86]) in liver related mortality (360). Accelerometry can provide objective evidence to monitor patient's PA and progression therefore further studies utilising this in CLD patients and validation is required.

1.6.3.5 Isokinetic dynamometry

This a frequently used modality to assess muscle strength in sport and exercise medicine and research studies but is less well used in CLD studies (361). An early study found isokinetic measures of force (both concentric and eccentric movements) were impaired in both the upper and lower limbs of patient with alcoholic related cirrhosis and other causes of non-alcohol related cirrhosis suggesting this is due to advanced liver disease rather than psychomotor capacity or as a direct result of alcohol (362). A study used isokinetic dynamometry to measure maximal force (peak torque) in a clinical trial evaluating combined

resistance and endurance training in liver transplant patients (363). Due to the equipment, training and accessibility, it remains as limited resource.

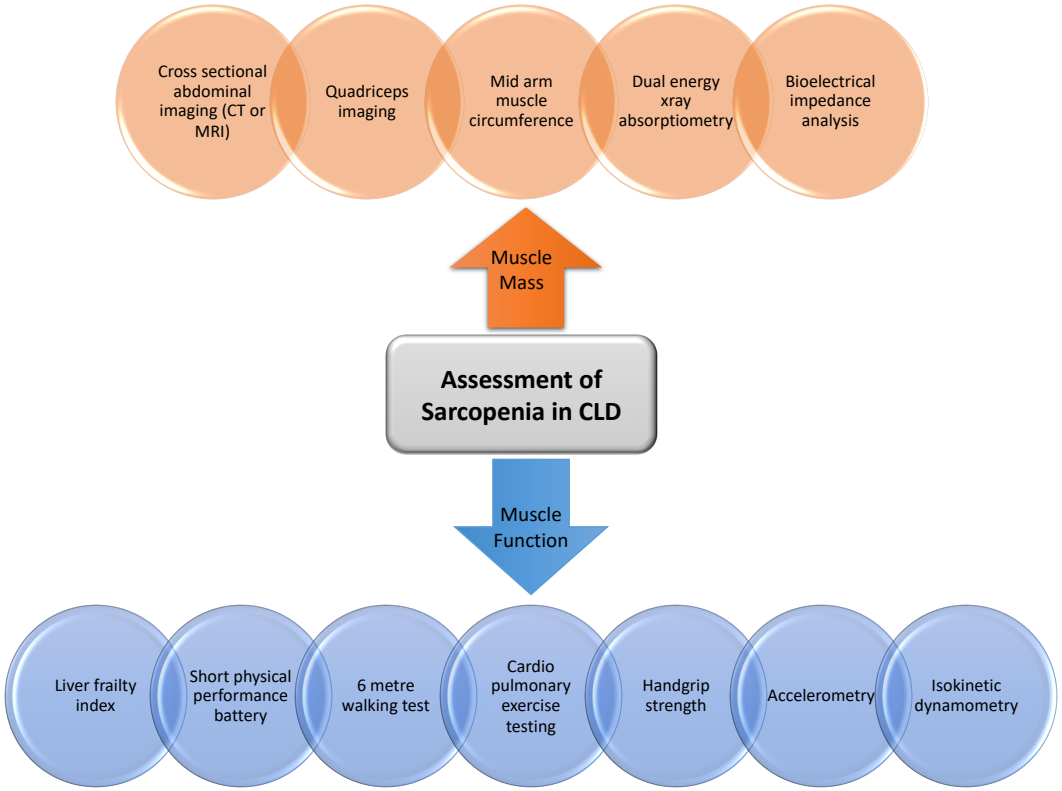


Figure 1-9: Overview of methods to measure muscle mass and function in sarcopenia in chronic liver disease

This figure highlights the methods of assessments that are used in clinical practice and have been trialled in research studies.

1.6.3.6 Quality of life

Sarcopenia is associated with poor HRQOL in the general population (364). In those with cirrhosis including HCC, both physical and role-social component summary scores (PCS and RCS) of SF 36 QOL questionnaires were lower in those with sarcopenia compared to those without. Sarcopenia was identified as an independent risk factor (OR: 4.64, P=0.016) for low

RCS (364). HGS has been associated with HRQOL domains such as physical function and PCS (365). Exercise is found to improve QoL in community dwelling populations (366) and therefore the role of QoL in CLD and sarcopenia, whilst not yet fully established, remains a target for further research and treatment.

1.7 Treatment of Sarcopenia in Liver disease

The treatment of sarcopenia requires a multi-faceted approach. The main targets for treatment are described below.

1.7.1 Lifestyle

All patients with CLD and those on the LT waiting list, irrespective of aetiology, should be advised to abstain from alcohol and smoking. This is even more paramount in the 'frail' patient as a result of the compound effect of ethanol and/or smoking has on sarcopenia (367, 368). It is also important to optimise glycaemic control in patients with glucose intolerance and type 2 diabetes in the setting of LT, as it is an integral part of preventing and potentially reversing sarcopenia. Insulin resistance and the resultant hyperinsulinaemic state not only drive fat accumulation in the muscle (myosteatosis), but also reduce protein synthesis and increase catabolism, which together drive sarcopenia (298). This is particularly pertinent in the immediate post-LT setting, as the standard inclusion of prednisolone in the immunosuppression regimen not only promotes hyperglycaemia but causes atrophy of muscles as well (369).

1.7.2 Nutrition

Patients with CLD have an 'accelerated starvation' and are susceptible to rapid muscle catabolism with prolonged periods of fasting. A reduction in fasting times is imperative to combat accelerated starvation state (244, 370). Therefore, a regular eating pattern of 2-3 hourly meals and snacks should be encouraged, with emphasis on the importance of not missing breakfast after an overnight fast (226). Although the optimal snack content is not defined in the literature, a late-evening snack/before bedtime is recommended, with 50g carbohydrate and up to 20g protein commonly utilised (197, 219, 317, 371).

All patients with CLD, and ideally at the point of diagnosis of ESLD, should undergo a comprehensive nutritional evaluation in the form of anthropometry (MAMC, HGS, triceps skin fold thickness, calculated dry BMI) and accurate analysis of food intake. To date, several tools have been developed to assess food intake as described previously including food dairies and recall (81, 82). However, it still remains unclear in the literature which tool is the most accurate as a result of limitations in reproducibility, clinical availability and inaccurate reporting (especially in patients susceptible to encephalopathy) (80).

International guidelines, as recently as 2019, are however consistent in their recommendations that an individual's caloric and protein requirements must be met by frequent feeding, oral dietary supplementation and/or when indicated by (par)enteral routes (197, 227). The latter is particularly important in patients hospitalised with acute deterioration, who are unlikely to meet their caloric requirements.

In non-obese patients (based on estimated dry body weight) the recommended daily caloric intake is at least 35 kcal/kg/day, whereas this should be cautiously reduced by 500-800 kcal/day in patients with sarcopenic obesity, in order to promote reduction in excess adiposity (197). The precise caloric target to achieve this effectively and safely (i.e. losing adipose mass whilst preserving/ improving muscle mass) remains unresolved (227). This is therefore a critical research question moving forwards, especially in light of the rising prevalence of NAFLD and its co-existing metabolic syndrome in patients referred for LT (372).

One of the most influential nutritional changes in advice over the last decade has been the switch from active protein restriction (373) to the consistent need to obtain high levels of protein intake, irrespective of the severity of encephalopathy (197, 227). The current clinical practice guidelines recommend caloric energy intake of 25–35 kcal/kg/day in compensated cirrhosis (Child–Pugh A) and 30–35 kcal/kg/day in decompensated cirrhosis (Child–Pugh B–C) (226) In addition, protein intake of 1.2–1.5 g/kg/day in compensated cirrhosis, with this increasing up to 2.0 g/kg/day in more severe cases of decompensation (e.g. in regular paracentesis for refractory ascites or hepatic encephalopathy) (197) is recommended.

BCAA have been studied with promising results and while formal recommendations are pending, there appears to be strong rationale for their use in promoting muscle protein synthesis (197, 210, 227). The decision to formally advise vegetable-based (rich in branched chain amino acids (BCAA)) over animal-based protein (rich in aromatic amino acids (AA)) intake still requires further research. However, there does appear to be a rational argument for avoiding amino acids that are not metabolised by muscle (i.e. aromatic AAs may promote

encephalopathy) and targeting those that reduce serum ammonia (i.e. BCAA via glutamate-glutamine pathway) (374).

It is also important to address any micronutrient deficiencies (such as vitamin A, D, E and K) which can develop especially in cholestasis and to address zinc deficiency in the presence of dysgeusia (71).

1.7.3 Exercise

Physical inactivity strongly predicts mortality in many chronic diseases. It is a key driver of sarcopenia in cirrhosis. Exercise has been shown to improve aerobic exercise capacity (6MWD), muscle function (SPPB), muscle mass (CT) and, importantly, quality of life in mainly compensated and, studied to lesser degree, decompensated cirrhosis (361, 375). To date, no studies have studied survival benefit. In general, exercise programmes should consist of a combination of aerobic (3 days/week) and resistance exercises (2 days/week), performed to a moderate intensity (i.e. out of breath but able to talk) and ideally be remotely-monitored in the patient's own home (275, 375, 376).

Over the last 5 years, there has been a drive to reverse physical frailty and improve physical function/exercise capacity in patients awaiting LT. Indeed, more recently American Society for Transplantation set out a clear research agenda for exercise interventions in patients awaiting solid-organ transplantation including LT (219, 256, 377). The application of exercise training in patients awaiting LT has previously lagged behind that of other forms of solid-organ transplantation (i.e. lung, heart), which in part may have been due to anxieties surrounding acute increases in portal pressures and the potential for variceal haemorrhage (378).

Reassuringly, however the Italian Sport Diet study of 50 patients with compensated cirrhosis highlighted that 16-weeks diet and exercise intervention was both safe and significantly reduced portal pressures (379).

To date, eleven studies (5 RCT, 5 observational, 1 case report) have demonstrated that exercise improves anaerobic thresholds, peak VO_2, 6MWD, muscle mass/function and to a certain extent quality of life in patients with compensated and decompensated cirrhosis (276, 380-382). None of these studies investigated survival benefit and the majority were small (1-50 patients), focus on supervised, hospital-based aerobic exercise interventions (i.e. cycle ergometer) and largely excluded patients requiring a LT (i.e. MELD>12) (375, 376, 383).

Large geographical areas, impractical travel times and costs, ill health and family and/or work obligations tend to limit the applicability of supervised (2-3x weekly) hospital-based exercise interventions in patients awaiting LT (376). In light of these limitations, recent studies (albeit small pilot studies) have focused on the safety, feasibility and efficacy of home-based exercise programmes in patients awaiting LT (383-385). In particular, one UK-based study (n=18) highlighted safety, compliance and improvements in physical function (SPPB, daily step count, shuttle walk testing) whilst on the LT waiting list, following a 12-week home-based combined aerobic (walking) and body-weight resistance exercises (no additional equipment) (383). Even though large RCTs are still required, in general, a combination of a minimum of 12 weeks of aerobic (3days/week) and resistance exercises (2-days/week), performed at moderate-high intensity, should be recommended to optimise muscle health and physical performance status whilst awaiting LT (376). Ideally, programmes should be easily accessible (preferably home-based) and focus on each individual's barriers to exercise and baseline physical frailty.

Further research is also required to guide nutritional replacement both before and after exercise, as this data is currently lacking in patients with ESLD.

Following LT, several rehabilitation studies have demonstrated that predominantly supervised (only one home-based) aerobic exercise improves measures of sarcopenia, exercise capacity and reported to a lesser degree - quality of life (386-388). Similarly, to the pre-LT setting, these studies are small and heterogenous (timing, type, intensity), but highlight that combined aerobic and resistance exercises yield the most promising improvements.

1.7.4 Pharmacological treatment

Pharmacotherapy is another promising research area in sarcopenia, with potential targets including micronutrients, vitamin D (389), ammonia-lowering treatments (266), hormonal therapy (i.e. testosterone replacement in males (283)), myostatin inhibitors, and L-Carnitine (an amino acid required for fatty acid oxidation, reported to suppress muscle loss) (390).

1.7.4.1 Ammonia lowering agents

Due to the significant role of ammonia in driving sarcopenia in CLD, there is debate regarding the use of ammonia lowering agents such as Rifaximin or l-ornithine l-aspartate (LOLA) to treat sarcopenia. A pre-clinical in vivo rodent study treating rats with 4 weeks of oral rifaximin and LOLA, demonstrated increase in lean body mass, improved grip strength, higher skeletal muscle mass and diameter and an increase in type 2 fibres in treated rats compared to controls (391). Whilst it is conceivable that ammonia lowering agents can help improve sarcopenia, clinical trials remain awaited (392).

1.7.4.2 Testosterone

There is only one RCT evaluating the effect of testosterone treatment in CLD. Sinclair et al tested intramuscular testosterone treatment in men with sarcopenia and cirrhosis and found higher appendicular lean mass in testosterone-treated subjects (283). This highlights testosterone as a potential treatment which need further research (393).

1.7.4.3 Vitamin D

As a fat-soluble vitamin, vitamin D deficiency is prevalent in CLD, affecting two thirds of those with cirrhosis. A Cochrane review examined the benefits and risk of vitamin D supplementation in liver cirrhosis but found no significant difference in mortality or adverse events (394). Both ESPEN and EASL recommend treating all CLD patients with vitamin D deficiency (197, 317). There are no studies yet investigating vitamin D supplementation in patients with cirrhotic sarcopenia (395). However a systematic review investigating the effects of vitamin D on muscle mass and strength demonstrated an increase in muscle strength but not mass (396), supporting other evidence of a positive correlation between vitamin D supplementation and muscle function (397).

1.7.4.4 Myostatin inhibitors

There has been a lot of pre-clinical interest in myostatin antagonists (398) and direct mammalian target of rapamycin complex 1 (mTORC1) (279) activators, which play key roles in protein synthesis and skeletal muscle mass. Despite provisional murine/primate studies that have highlighted that myostatin antagonism results in increased muscle mass and

performance, these interventions are likely to require extensive ‘mechanistic’ pre-clinical study and reproducible human clinical trials before they can be applied to routine clinical practice (398).

1.8 Thesis Aims and Objectives

Chronic liver disease has a significant impact on the homeostasis of muscle. The ongoing catabolic state that exists in those with liver disease may drive reductions in muscle mass, performance and function (71). As the definition of sarcopenia within liver disease remains an evolving entity, this can influence the measures of muscle mass and function utilised to assess sarcopenia in this cohort. The main hypothesis of this thesis was that muscle evaluation in chronic liver disease requires a multi modal approach and utilisation of both muscle mass and functional measures. Furthermore, in addition to evaluating the assessments modalities of muscle mass and function, the underlying mechanisms driving muscle compromise in those with liver disease remains an area for further exploration.

The aims of this thesis are:

- To evaluate the measure of muscle mass and function (strength and performance) in those with ESLD through a unique multi modal assessment to identify key considerations and methodologies when evaluating muscle health in this cohort.
- To assess the correlations of the truncal, upper and lower limb modalities uses to evaluate muscle mass and function.
- To compare the differences In the measures of muscle mass, quality, strength and performance in those with ESLD compared to an age sex matched healthy control cohort.
- To define clinical muscle phenotypes of muscle wasting in those with ESLD to identify the potential mechanistic driver of sarcopenia and frailty in this cohort.

The aims will be achieved through the following objectives:

To design and undertake a prospective, observational case-controlled study [Chapter 2] of adult patients with ESLD (with age-sex matched controls) consisting of a multi-modal assessment of muscle mass (MRI, Ultrasound, MAMC), strength (isokinetic dynamometry), function (LFI, SPPB) and muscle biology (muscle biopsy, serum cytokines/steroid analysis) in patients with ESLD; in order to:

a) To compare different imaging and functional tools in the assessment of muscle health in ESLD [Chapter 3]
b) To compare muscle health/phenotypes in patients with ESLD versus age-sex matched controls [Chapter 4]
c) To understand the impact of liver disease severity, aetiology, age and sex on muscle health in ESLD [Chapter 4]
d) To evaluate the different clinical muscle phenotypes (i.e. mass-to-function relationships) in patients with ESLD [Chapter 5] and using the healthy controls to determine adequate/inadequate cut-offs for muscle mass and strength.
e) To investigate possible mechanistic drivers of these different muscle phenotypes In ESLD by utilising transcriptomics from muscle tissue, serum cytokines analysis (i.e. myokines) and steroid profiling in the cohort. [Chapter 5]

2. Chapter Two: Clinical Methods

2.1 Study Design

2.1.1 Patient selection

A total of 53 patients with ESLD who were being assessed for liver transplantation were recruited. An age-sex matched sample of 18 healthy individuals as a control cohort were recruited for comparison.

2.1.2 Sample size

As this was a novel observational prospective cohort study with deep phenotyping (experimental) to determine the mechanisms underlying sarcopenia, there was no clear prevalence of the sarcopenia confirmed in our population, therefore a sample size calculation was not applicable. It was calculated that 50 patients in an ESLD cohort, based on population data, would be sufficient for a Spearman correlation of ≥0.3 to reach statistical significance. Recruitment targets were also based on feasibility within the time frame of the study and based on the planned statistical analysis.

2.1.3 Standard of care treatment

Within the ESLD cohort, patients who were listed on a transplant waiting list would undergo prehabilitation (the process of enhancing functional capacity to enable the patient to withstand a forthcoming stressor (399) as part of their standard of care management . This includes physical activity and nutritional optimisation (197, 276, 400). All those who were assessed for LT were also reviewed by a dietician for nutritional optimisation.

2.1.4 Eligibility Criteria

Eligible patients were screened via the following criteria (Table 2.1). ESLD patients were selected from those with ESLD who underwent assessment for liver transplantation.

Table 2-1: Eligibility criteria for participants

	Inclusion criteria	Exclusion criteria
ESLD	A formal confirmed diagnosis of liver disease - some may have had a liver biopsy; however serological and radiological confirmation will be sufficient. Meeting criteria of liver cirrhosis including all Child Pugh scores from A-C as per British Association for the Study of the Liver guidance. Adults aged ≥ 18 years Able to confirm written consent to the study For muscle biopsy sampling (does not preclude patients from participating if they do not meet the below criteria) INR ≤ 1.6 Platelet count > 30	Refusal or lack capacity to give informed consent. Currently enrolled in an interventional trial with active treatment for their chronic disease condition. Previously undergone LT or biliary intervention in the CLD cohort. Underlying or active cancer. For Muscle biopsies only (able to continue in study): Obvious injury to both thighs. Active bleeding of site, pre-procedure, Abnormal observation parameters. Acute illness. INR > 1.6. Platelet count < 30. Anticoagulation which cannot be paused due to increased risk to pre-existing co-morbidity. For undergoing an MRI Pacemaker. Metal work inserted that is not MRI compatible or further information cannot be obtained.
Healthy Controls	Adults aged ≥ 18 years. Able to confirm written consent to the study. No co-existing chronic inflammatory condition, cancer or significant premorbid disease pathology. No suspicion or evidence of sarcopenia.	Refusal or lack capacity to give informed consent. Pregnancy. Any recent acute illness or surgery requiring significant treatment or hospitalisation (within last 12 weeks). Any systemic corticosteroid use or replacement.

	No previous transplantation.	For Muscle biopsies only: Obvious injury to both thighs. Active bleeding of site, pre-procedure. Abnormal observation parameters. Acute illness. INR > 1.6. Platelet count < 30. Anticoagulation which cannot be paused due to increased risk to pre-existing co-morbidity.

2.1.5 Participant recruitment

Participants with ESLD were recruited from hepatology clinics in the Liver Unit, Queen Elizabeth Hospital, Birmingham UK, and members of staff from the University of Birmingham or Queen Elizabeth Hospital Birmingham, were recruited as healthy control participants from January 2019 to May 2021, with closure of the study by 30th June 2021. Due to the SARS CoV2 pandemic, recruitment was halted from March 2020 until March 2021. All participants were provided information and written consent which was approved by the University of Birmingham Research Governance, Health Research Authority and West Midlands Solihull Research Ethics Service Committee Authority (REC reference: 18/WM/0167, ClinicalTrials.gov Identifier: NCT04734496) who approved this study. Additionally, all written information was reviewed by a Liver and GI patient and public involvement group. All participants had to demonstrate capacity to consent.

ESLD Cohort

All patients who attended the outpatient LT assessment clinic were screened on the eligibility criteria. Patients with any degree of muscle wasting or functional decline were invited to take part in the study. Invitations to participate in the study occurred via face to face interaction during clinic and distribution of patient information sheets (PIS) via post or email to eligible

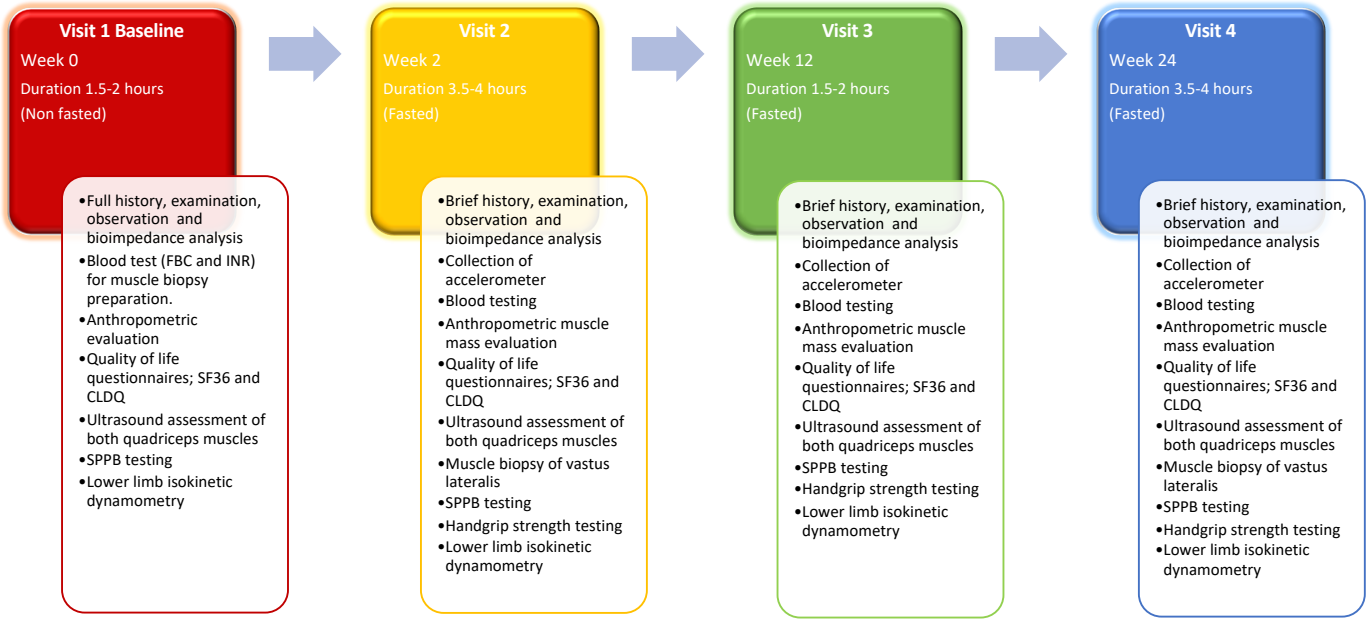
patients. The patients were telephoned up to 4 weeks after they received the PIS to discuss their potential participation. We recruited 53 patients with ESLD between January 2019 and March 2020. Seven patients in the ESLD cohort were lost to follow up and withdrew from participation. Seven Visit 4 appointments were cancelled due to the pandemic, in addition to cessation of further recruitment for this cohort.

Control Cohort

A provisional cohort of healthy control participants were recruited in March 2021 via poster advertising and through staff across the University of Birmingham. Ten participants were provisionally recruited however due to Sars-CoV-2 pandemic these were cancelled. Given the following restrictions and infection control measures, a further 18 participants were recruited from University of Birmingham and Queen Elizabeth Hospital Birmingham staff members.

2.2 Study Schedule and Method

This study entailed 4 visits spanning a 24-week period as described in this published protocol (401). Figure 2.1 provides an overview of the duration and assessment carried out for each visit. There was a two-week period surrounding each visit time point to accommodate the scheduling a visit to allow for potential liver transplantation, change of clinical appointments, unexpected illness or circumstances. Some visits were cancelled due to hospital admissions, liver transplantation or participant ill health. Visits were only carried out if the patient was well enough to attend.



*Short Form 36 questionnaire (SF36), chronic liver disease questionnaire (CLDQ), short physical performance battery (SPPB)

Figure 2-1: Summary of study visits

This figure with each timepoint and assessments performed at each visit.

Visit 1 -Week 0

During this non-fasted visit, formal written consent obtained for study participation and the muscle biopsy. A full history and examination, baseline blood tests anthropometric assessment (MAMC, MAC, TSF and HGS), quality of life questionnaires evaluation, ultrasound assessment of both quadriceps, lower limb leg strength and power was assessed via isokinetic dynamometry, short physical performance battery were performed. The details of each assessment are described further below.

Visit 2 -Week 2

During this initially fasted visit, a brief history and examination, blood tests, quality of life evaluation, anthropometric muscle mass measures (MAMC, MAC, and TSF) ultrasound assessment of both quadriceps were performed on each participant. For those participants

that consented, a muscle biopsy was performed whilst the participant was fasted. The fasting period was then ended with a food break. Lower limb leg strength and power was assessed via isokinetic dynamometry, short physical performance battery and hand grip strength were then assessed. Food diaries and worn GENEActiv® watches were collected (which were distributed to participants two weeks prior to their visit).

Visit 3 -Week 12

During this initial fasted visit, a brief history and examination, baseline blood tests anthropometric assessment (MAMC, MAC, TSF and HGS), quality of life questionnaires evaluation, ultrasound assessment of both quadriceps were performed. The fasting period was then ended with a food break. Lower limb leg strength and power was assessed via isokinetic dynamometry, short physical performance battery and handgrip strength were then assessed. Food diaries and worn GENEActiv® watches were be collected.

Visit 4 - Week 24

During this initially fasted visit, a brief history and examination, blood tests, quality of life evaluation, anthropometric muscle mass measures (MAMC, MAC, and TSF) ultrasound assessment of both quadriceps were performed on each participant. For those participants that consented, a muscle biopsy was performed whilst the participant was fasted. The fasting period was then ended with a food break. Lower limb leg strength and power was assessed via isokinetic dynamometry, short physical performance battery and hand grip strength were then assessed. Food diaries and worn GENEActiv® watches were collected.

2.3 Assessment Modalities

All participants underwent a full baseline history and examination at the start of the study. For each subsequent visit, an updated history and examination was performed. The following assessments were performed as described below.

2.3.1 Assessment of blood samples:

Blood was sampled using a BD vacutainer system. A maximum of 60ml of whole blood, via several different vacutainers was sampled. The blood samples were rested to clot for 30 minutes at room temperature. The samples were centrifuged at 1620 X g for 10 minutes (serum) and 438 X g for 8 minutes). The upper layers were collected and aliquoted into 1ml Eppendorf tubes for storage at -80 degrees. They were later thawed on ice for analysis. Further freeze thaw cycles were avoided. Routine blood samples for C reactive protein, ESR, baseline renal function, liver function, full blood count, clotting function, vitamin D, ferritin, folate, vitamin B12, thyroid function, lipids, and trace elements (zinc, selenium and vitamin A) were sent to the clinical pathology laboratories at Queen Elizabeth Hospital Birmingham for analysis (402).

2.3.2 Anthropometric assessment

Height was measured using a freestanding telescopic measuring rod. Waist circumference was measured using a tape measure at the narrowest part of abdomen around the naval, approximately midway between the lower ribs and iliac crest (403). The tape is pulled tight without indentation into the skin. Hip circumference was measured at the largest circumference around the buttocks, usually over the greater trochanters (403), and body mass index was measured. Waist to hip ratio (WHR) was calculated. Body mass index was calculated

A ‘dry’ body weight was calculated by subtracting the percentage body weight based on the degree of ascites or peripheral oedema as recommended by ESPEN and EASL guidance (197, 317) from the total body weight. Those with mild, moderate or severe ascites had 5%, 10% or 15% of their body weight deducted from their actual body weight. For those with peripheral oedema, 5% for mild and 10% for moderate oedema was deducted. Body mass index (BMI) was calculated by dividing weight (kg) / (height $(M)^2$). For the calculation of dry BMI, the estimated ‘dry’ weight as described above was substituted in place of weight.

A Tanita T5896 segmental body composition analyser was used to measure weight and for bioelectrical impedance analysis. The participant had to stand bare footed on the metallic foot plates on the base, and had to grip the metallic plated handles for approximately 30 seconds until instructed by the device. A readout including BMI, BMR, body fat, free fat mass, and total body water was obtained (60).

2.3.3 Muscle mass assessment

2.3.3.1 Mid Arm Muscle Circumference (MAMC)

The MAMC was calculated via first measuring mid arm circumference (MAC) and triceps skin fold (TSF) thickness. Firstly, the midpoint between the lateral edge of the acromion and olecranon process of the radius on the dominant arm was identified. MAC was measured at the midpoint mark, ensuring the measuring tape was even against the skin (without being taut). This was repeated twice, and the mean result calculated. Following this, TSF was calculated via a Harpenden calliper at the same midpoint as above. This process was completed twice, and an average of the two measurements was recorded. MAMC was calculated using equation 1.

$$MAMC = MAC - (\pi \times \left(\frac{TSF}{10}\right))$$

Equation 1: Calculation of Mid arm muscle circumference (MAMC). MAC=mid arm circumference, TSF=triceps skin fold. (404-406).

2.3.3.2 Quadriceps ultrasonography

Sagittal ultrasound images of the vastus lateralis (VL) were obtained as previously described utilising a portable ultrasound machine (MyLab Alpha, Esaote, Genoa, Italy) attached with a 3-13 MHz, 4.7 cm linear array transducer (SL1543, Esaote, Genoa, Italy). (Figure 2.2). Measurements were performed on the dominant leg with the participant lying supine, following a 10minute rest period. Scans were acquired at 50% of femur length (defined as 50% of the distance between the greater trochanter and midpoint of the patella) (407). In addition, the mid sagittal axis of the VL was obtained (identified as the mid-point between the medial and lateral borders of the muscle, as assessed by ultrasound). Thus, an exact relative point was obtained for image acquisition. Images were analysed offline via a semi-automated image J macro tool (Simple Muscle Architecture Analysis) (408-410) which allowed for the estimation of VL fascicle length and pennation angle. Analysis was completed in triplicate and the mean average values for fascicle length and pennation angle were acquired and consequently also utilised for the estimation of effective physiological cross-sectional area (as detailed below).

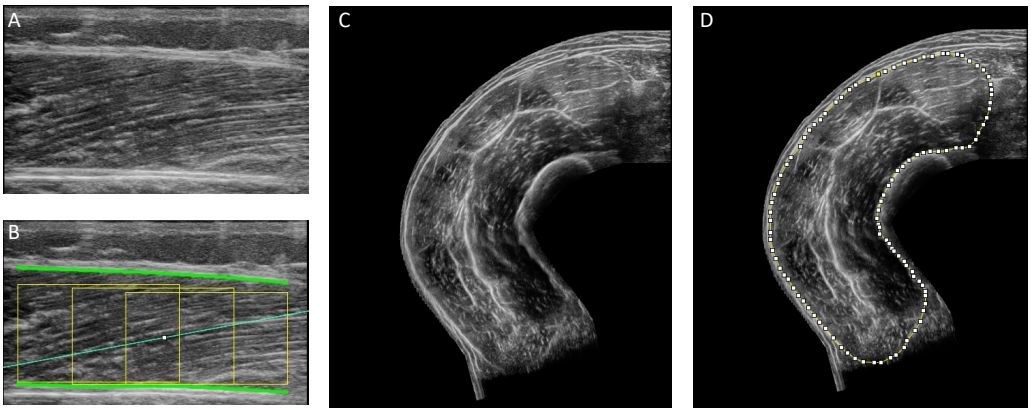


Figure 2-2: Quadriceps ultrasound

This shows the images acquired by quadriceps ultrasonography. Image A shows a sagittal image of the vastus lateralis muscle at the mid-point of the femur length. Image B highlights the aponeuroses are demarcating the muscle (parallel light green lines) with an individual fibre (dark green line) highlighting a selected fibre to calculate the fascicle length and subsequent pennation angle. Image C demonstrated a cross sectional view of the quadricep muscle attained by an extended field of view ultrasound, which is manually segmented on image D.

Vastus Lateralis Muscle Volume and Physiological Cross-sectional Area

VL muscle volume was estimated for the purpose of calculating VL effective physiological cross-sectional area (PCSA) and was done in similar fashion to quadriceps muscle volume, i.e., anatomical cross-sectional area (ACSA) slices obtained along the muscle at 4cm intervals via Horos software (version 3.3.6). However, in contrast to the quadriceps muscle volume, ACSA slices are obtained across the full length of the VL rather than the restricted ROI1. VL muscle volume was consequently estimated via the truncated cone equation (equation 1). VL PCSA was consequently calculated as VL muscle volume divided by VL fascicle length as previously reported (9, 411). In order to correct for force transmission to the tendon and provide the

most accurate measure of muscular torque production, effective PCSA ($PCSA_{eff}$) was calculated by multiplying PCSA by the cosine of VL pennation angle (411) .

2.3.3.3 MRI quadriceps

A MRI of the quadriceps was performed following risk assessment and a safety checklist to exclude any contraindication to MR imaging. All image results underwent formal reporting by Consultant Musculoskeletal Radiologist. The methods of analysis for the MR measures are described below:

Quadriceps Muscle Volume

Images were collected via a 3T Cobalt MRI scanner with T1-weighted turbo spin echo sequence with repetition time 600ms, echo time 15.2ms, field of view 512 x 512 mm and 1cm slice thickness with no slice gap. Offline manual segmentation of the quadriceps from the dominant leg was completed by a single investigator via Horos (version 3.3.6). The calculation of quadriceps muscle volume utilised ~7/8 anatomical cross-sectional area (ACSA) slices with 4cm interslice thickness from a restricted quadriceps region of interest. Similar to previous work, both proximal and distal extremes of the quadriceps were omitted from analysis to increase accuracy (412). The proximal limit was identified as the appearance of the lesser trochanter and the distal limit as 20% above the proximal aspect of the patella (413). It has been previously shown that the use of 4cm interslice thickness maintains accurate estimations of muscle volume within this restricted region of interest (413) Significantly, any sections which were identified as adipose tissue or non-contractile tissue were not included in muscle ACSA analysis. Consequently, muscle volume was estimated via the truncated cone equation (equation 2).

$$MV_{cone} = \Sigma\left(\left(\frac{1}{3} \times slice\ thickness\right) \times \left[CSA_n + \sqrt{(CSA_n \times CSA_{n+1})} + CSA_{n+1}\right]\right)$$

Equation 2: *Truncated cone calculation of muscle volume, whereby both the current (CSA_n) and sequential (CSA_{n+1}) ACSA are required for the estimation.*

Quadriceps muscle volume was normalised to height squared for each individual such that muscle volume is presented as quadriceps muscle index (cm^3/m^2).

Quadriceps Mid-ACSA

In addition to the ACSA values obtained for muscle volume estimation, quadriceps ACSA at 50% of femur length (distance between greater trochanter and medial patella), was also calculated for the dominant leg. An oil capsule was fixed at this 50% point prior to MRI (Figure 2.3). Post-acquisition, the slice representing 50% femur length (mid ACSA) was identified via the appearance of the capsule and the quadriceps ACSA was manually segmented at this slice.

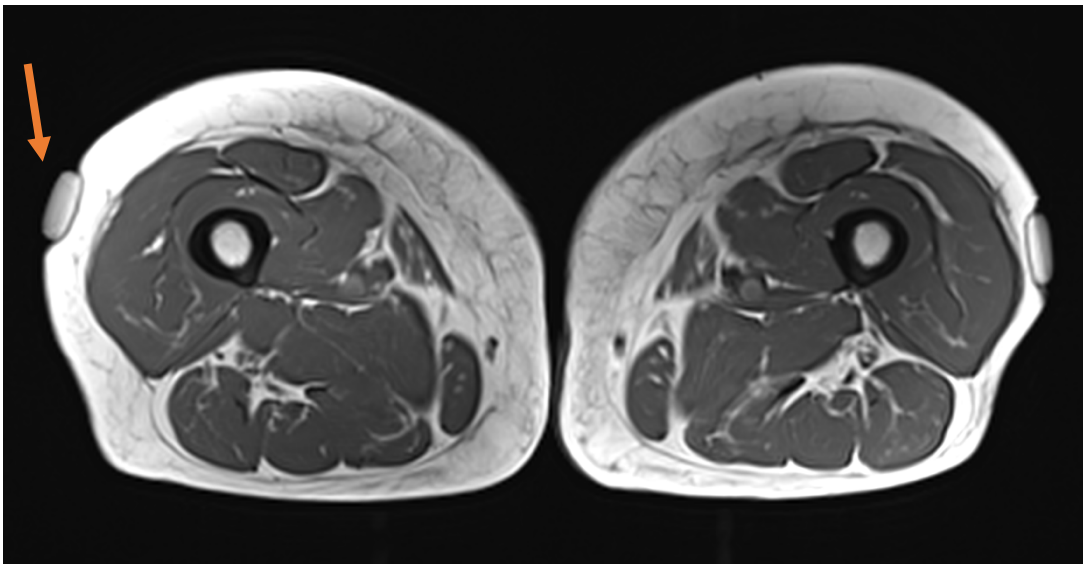


Figure 2-3: Cross sectional image of the thigh

On this cross sectional muscle of the thigh, the attached fish oil capsule (red arrow) demonstrate the 50% midpoint of the femur length (distance between the greater trochanter and medical patella) on MR imaging.

L3 Skeletal Muscle Index

Muscle CSA at the L3 level was manual segmented via Horos software (version 3.3.6). Muscles at the L3 region included the psoas, erector spinae, quadratus lumborum, transversus abdominus, external and internal obliques, and rectus abdominus. Consequently, L3 SMI was calculated by normalising the L3 muscle CSA to height^2 of each patient (cm^2/m^2). This analysis was only available for n=32 in the ESLD group.

Intermuscular Adipose Tissue

Intermuscular adipose tissue (IMAT) was estimated via the two-point Dixon sequence method (414) with offline analysis via Horos software. IMAT was calculated on the mid-quadriceps ACSA as previously identified. Specifically, manual segmentation of the quadriceps ACSA was completed on both the 'fat only' and 'water only' fractions and consequently the mean signal intensity of the quadriceps ROI was calculated for each fraction. IMAT percentage was then calculated as per equation 3 and the data presented herein are generated from the dominant leg.

$$Quadriceps\ IMAT\ (\%) = \left(\frac{Fat_{\ SI}}{(Fat_{SI} + Water_{SI})}\right) \times 100$$

Equation 3: Estimation of quadriceps IMAT calculation via two-point Dixon technique and signal intensity (SI) of fat only and water only fractions.

Specific force

Specific force (i.e., force per unit area of muscle) was calculated as the peak torque produced during the isokinetic assessment divided by quadriceps ACSA obtained at 50% of femur length

as identified by the oil capsule (equation 4). For the calculation of specific force, quadriceps ACSA was normalised to IMAT percentage, such that a more accurate estimation of contractile tissue was considered. This value was calculated for the non-dominant limb as per the reasons previously explained. For the purpose of specific force, both quadriceps ACSA and IMAT percentage were also calculated for the non-dominant limb utilising the same methodology as described above.

$$Specific\ force = \frac{Isokinetic\ torque}{Quadriceps\ ACSA\ \times\ (1 - IMAT\ \%)}$$

Equation 4: Calculation of specific force. IMAT= intermuscular adipose tissue.

2.3.4 Muscle strength assessment

2.3.4.1 Maximal handgrip strength

This was assessed with both the dominant and non-dominant side via a hand grip dynamometer (Takei, 5401 GRIP-D). Participants were asked to stand up straight and hold the hand grip dynamometer directly by their side and encouraged to exert their maximal grip strength for 5 seconds (60, 415). This was repeated for 3 attempts with a rest of 30 s between attempts. Maximal handgrip strength was defined as the highest value from the 3 attempts. Average HGS for each limb were also calculated.

2.3.4.2 Isokinetic Knee Extensor Strength

Measurements of unilateral maximal isokinetic torque for the knee extensors was conducted via isokinetic dynamometry, on the Biodex Medical System 3 (Biodex Medical Systems, New York, United States). Prior to assessment, participants underwent a short warm up and were

familiarised with the equipment and protocol. Participants were seated and tightly secured via straps across the test limb, the hips, and the chest; thus, isolating the test muscle group. The dynamometer arm was tightly fixed to the participant's ankle and maximal range of motion was assessed. The assessment protocol consisted of 5 consecutive maximal isokinetic leg extension contractions (60 deg.s^{-1}) of the dominant and non-dominant leg on Visit 1 and 3, but limited to non-dominant limb on Visits 2 and 4 as muscle biopsies were collected from the dominant leg prior to this assessment as part of this study (401). Maximal isokinetic torque was defined as the highest recorded torque value during the 5 completed contractions.

2.3.5 Muscle function assessment

2.3.5.1 Physical Activity and Sleep (accelerometry)

In order to assess habitual physical activity, participants were provided with a GENEActiv® accelerometer (Activinsights, Cambridge UK) which was worn on the non-dominant hand for a minimum of 3 days and maximum of 14 days prior to the visit. These devices allowed measurement of body movements over three orthogonal axes (vertical, horizontal right-left, and horizontal front-back axes). The accelerometers were programmed to sample at a frequency of 10Hz and the data were stored in 5-second (s) epochs. The three dimensional raw data was expressed in gravitational equivalent units called milli-gravity (mg, where 1000mg=1g=9.81m/s2). They were programmed and data downloaded in binary format using GENEActiv® PC software (version 3.1). Extracted GENEActiv®.bin accelerometer files were extracted, processed and analysed with an open-source R-package, GGIR (Version 2.5-0, http://cran.r-project.org) (416-418). GGIR was developed for GENEActiv accelerometers and uses raw acceleration ENMONZ values (i.e. Euclidian norm minus one with negative

values set to zero) with validated cut-points to determine intensity of physical activity, in addition to the positioning and elevations of the wrist. Detection of non-wear and sustained abnormally high values, autocalibration, using local gravity as a reference, and the 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 (mg) averaged over 5-s epochs were included in the signal processing. Participants were excluded if there were <3 days of valid wear (defined as >16 hours/day), if wear data were not present for each 15 minute period of the 24 hours cycle, or if the post calibration error was greater than 10mg. Threshold were set for inactivity (<40mg), light, moderate/ vigorous activity (>100mg) (416-418). For sleep detection, GGIR identified periods of sustained inactivity where there is a smaller change in arm angle than a predefined threshold (419). Data were available for only 30 ESLD patients and 17 control participants.

The measures used are summarised below:

Overall daily activity (mg); the average acceleration (volume of activity) as calculated for each valid day and averaged across all valid days.

Intensity gradient: the intensity distribution of activity which was calculated for each valid day and averaged across all valid days.

MVPA (>100mg) (mins per day): the average time spent at moderate vigorous physical activity of >100mg.

Days with 30 mins walk (>100mg, n): the average number of days with a 30 minute walk at a moderate-vigorous threshold of intensity (>100mg).

Days with 15 minute walks (>100mg, n): the average number of days with a 15 minute walk at a moderate-vigorous threshold of intensity (>100mg).

Total inactive time (mins) defined as time accumulated below a previously defined threshold of inactivity (<40mg).

Total inactive days: the average number of days.

Sleep window length (hours): the total time spent in bed (calculated as the difference between sleep onset and waking time).

Actual sleep time (hours): the average accumulated nocturnal sustained inactivity bouts during sleep period).

Sleep efficiency (%): the ratio between total sleep time and total time in bed.

2.3.5.2 Short Physical Performance Battery (SPPB)

This consisted of three tests described below as described by Guralnik et al (420, 421). This is scored out of a maximum of 12 points, with a maximum of 4 in each domain.

Balance: participants were asked to stand unaided with their feet placed together for 10 seconds, without moving their foot position. They were then asked to stand in a semi tandem stance, and asked to hold this for 10 seconds. Finally, they were asked to stand in a tandem stance for a further 10 seconds.

Gait speed: a 2.44 metre distance was defined with markers at each end. The participant was asked to walk from one end to the other at their usual pace. The time taken was recorded. This was repeated again. The lowest time taken to perform the walk was recorded

Chair stands**:** Participants were asked to sit back fully in a chair (42cm height) and cross their arms across their chest. Participants were then asked to complete 5 sit to stands in the quickest time possible, ensuring they stood up and sat back fully on each repetition. The time taken to complete 5 full sit to stands was recorded.

2.3.5.3 Liver frailty index

This was calculated using the hand grip strength, chair stand time and balance results from SPPB. It was calculated using the Liver Frailty index tool https://liverfrailtyindex.ucsf.edu/ (273).

2.3.6 Muscle tissue evaluation

A biopsy of the vastus lateralis via an aseptic technique using a Bergstrom technique.

Two weeks prior to their muscle biopsy visits, a medically trained clinical research fellow checked all medications including any antiplatelet, anticoagulation, insulin, or oral hypoglycaemic medications. No biopsy procedure would occur if pausing of any medication could incur significant risk (e.g., warfarin for venous thromboembolism). However, when feasible, medication was paused for a minimum of 3 days in advance of the biopsy visit, in accordance with drug half-life and national guidelines for gastroenterology endoscopic procedure (422) In addition to anticoagulation and antiplatelet medication, insulin use was screened and participants were in advance with advise on adjustment of their insulin doses the day prior to and day of the biopsy. This followed national guidelines for GI endoscopic procedure and radiology procedural guidelines (422-424) To ensure capacity and that the patient understood the procedure, consent was obtained on multiple occasions (prior to the visit, on arrival, and pre-biopsy).

On the biopsy visit, a stringent safety checklist was utilised to ensure that every patient was adequately risk assessed. A Local Safety Standards for Invasive Procedures (LocSSIP) checklist [similar to a World Health organization (WHO) checklist used in surgery] for performing the muscle biopsy procedure, was devised. The LocSSIP reconfirms capacity, consent, notes any

allergies and medications, as well as confirming any medications that have been temporally paused for the procedure. A pre-biopsy check of INR and platelets were measured, whereby a cut-off of INR ≥ 1.6 (normal value < 1.2) and platelet count ≤30 × 10^9/L (normal range 50–450 × 10^9/L) would have contraindicated a biopsy; in concordance with national and local radiology guidance for procedures entailing a similar risk profile (422-424). If all safety concerns were satisfied, a point of care blood sugar (BM) check occurred, as those with diabetes and CLD are at an increased risk of hypoglycaemia due to fasting and liver dysfunction. The cut-off point of <4.0 mmol/L was set as contraindication for muscle biopsy. Finally, ultrasonographic assessment of the vastus lateralis occurred during each visit and as such helped identify the optimal muscle biopsy site. The captured images were also interpreted for VL size and the degree of subcutaneous adipose tissue and hence the depth from skin to muscle tissue. The scans also enabled assessment for any aberrant vessels, or potential complications. Although the procedure itself was "blind," the biopsy site was visually assessed for morphology and any possible contraindications. In addition to those mentioned above, other contraindications at the pre-biopsy check stage included any pre-existing swelling, infection or injury surrounding the proposed biopsy site or lower limb, systemic acute illness or haemodynamic instability. The protocol was to immediately stop the procedure if concerns were raised (from either the operator or patient) (425)

Muscle biopsy technique: The participant was fasted for 6 hours prior to the procedure. A small area of skin overlying the outer thigh was cleaned with iodine solution. 5-10ml of 1% lignocaine was infiltrated into the subcutaneous adipose tissue and down to the muscle. After adequate anaesthetic, a small incision was made in the skin (approximately 5-7mm in length). A needle was inserted into the muscle and a small amount of muscle was sampled using a

well-described technique with a Bergstrom needle (426). A maximum of three passes were performed. The incision was closed using steri-strips adhesive dressings. A further small dressing was placed over the biopsy site. Pressure and an ice compress was be applied to the area for 10 minutes by hand. A pressure bandage was applied for some participants to decrease the risk of bruise formation. Participants were asked to keep this area dry for at 3-5 days, and received after care advice including contact details for the research team, if needed.

2.4 Statistical Analysis Plan

All quantitative data was entered into a purpose-designed database. It was exported for statistical analysis in both Prism Graph Pad version 9 version 2020, and R statistical software.

Data were tested for normality using Shapiro-Wilk or Kolmogorov Smirnov test. The appropriate parametric (one-way ANOVA for unpaired data, independent or paired t-test) or non-parametric test (Kruskal Wallis ANOVA for unpaired data, or Wilcoxon paired test for paired data). Correlation and relationship between variables were evaluated with a Pearson rank correlation test for parametric distributed data or Spearman's correlation test for non-parametric distributed data. A p value <0.05 was considered to be statistically significant. All additional analyses will be further described in each chapter.

Both quality of life and nutritional intake data were not incorporated within the remit of this thesis therefore the methods of analysis have been excluded. All additional methods that have been used in this thesis, have been incorporated into each specific chapter.

Furthermore, data from Visit 3 and 4 were not included within the remit of this thesis, and the following chapters solely focus on data from Visit 1 and 2 only.

3. Chapter Three: Measures of muscle mass and muscle function in End Stage Liver Disease

3.1 Introduction

End stage liver disease (ESLD) has a significant impact on the homeostasis of muscle, with observed reductions in muscle mass, performance and function due to an ongoing catabolic state (71). The definition of sarcopenia within ESLD remains an evolving entity and differs from the definition of age-related sarcopenia set by the European Working Group on Sarcopenia in Older People (EWGSOP). These latter guidelines define sarcopenia as reduced muscle strength with low muscle mass or quality and in severe cases low physical performance (59). Most recently, practice guidance by the American Association for the Study of Liver Diseases (AASLD), has formulated operational definitions of sarcopenia and frailty, evolving from theoretical definitions that exist. The AASLD definition of sarcopenia is the phenotypic presentation of muscle mass loss, whereas frailty is used to describe elements of muscular contractility, physical performance and function (256).

The most commonly used measure for the assessment of muscle mass in this clinical cohort remains the L3 skeletal muscle index (SMI) via CT or MRI cross-sectional imaging which is recommended by the North American FLEXIT (Fitness, Life Enhancement, and Exercise in Liver Transplantation) Consortium which validated cut-offs for L3 SMI of < 50 cm2/m2 in men and < 39 cm2/m2 in women (219, 325). There are evolving studies utilising bedside investigations such as ultrasonography for assessment of muscle mass. Indeed, Tandon *et al* have shown that ultrasound measures such as quadriceps muscle thickness have strong associations with

sarcopenia in patients with cirrhosis when compared to L3 SMI (222). Futhermore, there has been research into the use of larger muscle groups such as quadrcieps to evauluate muscle mass rather than truncal or upper limb muscle groups (427). This is now being investigated in those with ESLD, rather than the use of L3 SMI.

Aside from lower limb measures of muscle mass, mid-arm muscle circumference (MAMC) is an inexpensive and simple test of the upper limb muscle status. When performed by trained individuals, MAMC has good intra and inter-observer agreement, highlighting the reproducibility of the measure (336) and has been shown to predict mortality in patients with cirrhosis and those after LT (337). Bio impedance analysis (BIA) and dual X ray absorptiometry (DXA) have also been used to measure muscle mass in cirrhosis, by estimating fat free mass and lean tissue mass respectively. These tests are reproducible in populations such as older adults (59), however validity in patients with ESLD remains unclear. DXA and BIA cannot differentiate between lean muscle mass and body water therefore this can lead to inaccuracies in patients with ascites, hydrothoraces and peripheral oedema, which are commonly present in those with ESLD (428). It is also noteworthy that DEXA has some degree of radiation, although considerably less than CT.

Frailty, as defined above and in the context on ESLD, is typically assessed through a variety of muscle function and performance measures (256). Handgrip strength (HGS) is the most readily used assessment of upper limb muscle strength due to its ease to perform, the ability to serially measure and assess progression/regression, as well as possessing the strongest evidence base in ESLD (197). Subsequently, HGS is currently recommended by both recent international guidelines (European Association of Study of Liver (EASL); European Society for

Clinical Nutrition and Metabolism (ESPEN)) in the assessment of all patients with cirrhosis and liver failure (197, 227). HGS has been shown to be a better predictor of adverse clinical outcomes than measures of muscle mass (355) and MELD (225) and it correlates strongly with lower limb extremity muscle power (356). Aside from measures of upper limb strength, assessments of lower limb muscle function and performance include chair stands, as well as the 6-minute walk test (6MWT). Chair stands are an easy to use, reproducible and quick measure of muscle performance. In fact, Lai *et al* showed that chair stands in conjunction with HGS, are a strong predictor of mortality for those with ESLD on a liver transplant waiting list (AUROC =0.72) (274). The 6MWT is a continuous scale of distance based on a sub-maximal exercise test used to assess aerobic capacity. Cut-offs for distance have been described in relation to all-cause mortality (347, 348). The largest study to date of 213 clinic patients with ESLD awaiting LT highlighted that a distance of <250 metres resulted in a 2-fold increased risk of mortality (348). Challenges with 6MWT are that it requires a flat 30 metre walk way (which isn't always practical in out-patient setting) and can be influenced by learned behaviour (i.e. improvements with patient familiarity of testing). Isokinetic leg strength, which has been widely used in other fields as a measure of muscle strength and function, is an evolving medium in ESLD. Isometric knee extension has been used by Lai *et al* assessing performance-based measures and their association with frailty in patients with ESLD (429) and it demonstrated good correlation to other performance-based measures such as HGS, chair stands and 6MWT. However, the use of leg strength measures require further validation in this field.

In addition to the individual assessments named above, there are also composite tests such as short physical performance battery (SPPB) testing and the liver frailty index (LFI) which

combine function/performance and strength modalities. The SPPB consists of chair stands, gait speed and balance (each scored out of 4; with a maximal total score of 12) and is associated with overall mortality in a LT cohort, with a cut-off < 10 increasing the odds of mortality by 2.5 (352). It is, however, limited in patients with higher levels of baseline physical function, as it has a ceiling score of 12 out 12, which means the true impact of interventions may be overlooked in these individuals as demonstrated by Williams *et al* (430). LFI includes the assessment of three performance measures: HGS, time to complete 5 chair stands and time held balance positions (side by side, semi tandem and tandem stance) (274). The LFI has established cut-offs to define robust, pre frail and frail performance states (scores of <3.2, 3.2-4.3 and ≥4.4 respectively) is a reliable test and is well-validated in cirrhosis and ESLD (346). Another, composite measure is physical activity which can be measured by accelerometry, through wrist worn or body worn accelerometers. They can measure a number of variables including step count, levels and durations of physical activity e.g. light, moderate, strenuous, and inactivity, which can be used as a surrogate for sedentary time. A solitary study by Dunn *et al* of 53 patients observed lower levels of step counts in those with ESLD awaiting liver transplantation compared to healthy adults (359). This newer mode of measuring function is gaining further momentum in evaluating physical activity, and monitoring response to intervention.

With ESLD, it is important to acknowledge that measures of muscle function and strength can be negatively affected by complications of liver disease, such as portal hypertension (ascites, variceal haemorrhage), peripheral oedema and hepatic encephalopathy. Ascites and peripheral oedema can limit tests such as chair stands, balance and gait, reducing the performance. Hepatic encephalopathy can impair a patient's ability to accurately follow

command and adhere to instruction, resulting in a potential underperformance. It also has a negative impact on compliance (physical activity, nutrition, medications) and remains a significantly disabling symptom of decompensation (431). Whilst there have been previous concerns regarding the risk of exercise acutely elevating HVPG and increasing subsequent risks such as variceal bleeding, it has been shown in a small clinical trial by Macias-Rodriguez *et al*, that HVPG was decreased with a 14-week aerobic exercise programme (432). At present, there is no evidence to suggest that measuring muscle function through the above methods increases HVPG. When measuring muscle function, steps can be taken to diminish the impact of ascites and HE, including use of diuretics and paracentesis to improve fluid shifts, and use of treatments such as Rifaximin and lactulose, to reduce and control HE.

There has been research into the cross correlation of measures of muscle mass and function and the relationship between them. It is important to note that work in other fields, such as cancer cachexia has suggested that the choice of muscle group assessed may have different relationships to various functional outcomes (427). HGS has a known correlation with conventional measures of muscle mass, including CT-SMI and DXA. Piratisuth *et al*, recently consolidated this in a retrospective study of patients with cirrhosis, by demonstrating low HGS (using male < 26 kg, female < 18 kg cut-offs) correlated to low L3 SMI (male < 42 cm2/m2, female < 38 cm2/m2) via local cut-offs ($r = 0.81$, $p < 0.001$) (433). Additionally, Chan *et al* found a correlation between HGS in older adults with both upper (males $r = 0.576$, $p < 0.001$; females $r = 0.262$, $p < 0.001$) and lower extremity muscle mass via DXA (males $r = 0.532$, $p < 0.001$; females $r = 0.364$, $p < 0.001$) (434). However, the relationship of muscle mass and function remains less researched within ESLD, including the relationships of upper and lower limbs and truncal mass measures to muscle function variables. Furthermore, with

the exception of chair stands, there has been very little focus on the lower limb mass and functional measures in those with ESLD. This is particularly pertinent as lower limb muscle health is essential for functional independence of individual (435). Further studies are therefore required to evaluate the degree of correlation between these methodologies of mass and function to aid confirmation of the validity and accuracy of these measures within an ESLD population.

Therefore, the aim of this chapter was to evaluate the correlation of each modality used to measure muscle mass, strength and performance within ESLD, comparing the measures to commonly used measures such as L3 SMI and HGS. An additional aim is to investigate limb specific measures of muscle mass and function and evaluate their correlates .

3.2 Methods

3.2.1 Study population

Chapter 2 outlines the study schedule including each visit, the time frame and the assessments measured at each visit. Fifty-three participants with ESLD were recruited to this study with eighteen age and sex matched healthy control participants. Of those recruited, 42/53 participants attended visit 2, whereby in-depth muscle phenotyping was performed. Eleven participants did not attend their visit 2 due to various factors including 4 patients with ill health, 2 patients who has undergone liver transplantation and 5 patients withdrawing from the study after their first visit (Figure 3.1). Of these 42 participants, only 39 participants underwent an MRI (due to technical and safety issues encountered at the time) (Figure 1), and were used in this analysis. Visit 1 and 2 took place approximately 14 days apart. Any missing data from this Visit 2 for blood results, height, and weight, were utilised from those

taken at Visit 1. Of note, all 18 healthy volunteers underwent MRI and the protocolised tests at one visit.

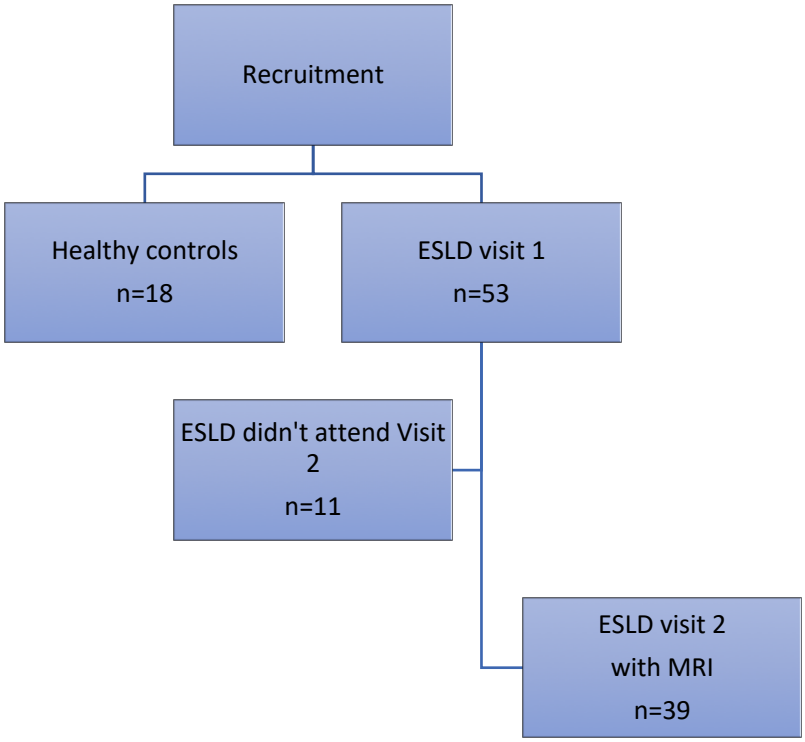


Figure 3-1: Flow chart of the recruitment for end stage liver disease (ESLD) and healthy controls

3.2.2 Assessment modalities and methods

Chapter 2 provides detailed analysis of all muscle assessment modalities performed as well as an in-depth description of methodology. However, for the purposes of this chapter the following assessment modalities that were included can be seen in Table 3.1. The data included for muscle mass and function variables were collected from the Visit 2. Accelerometry data was obtained in 30 of the 39 participants. Whilst this remains an emerging field, developments have recently been made by our own group, where investigations into the use of MRI to assess quadriceps muscle mass in those with ESLD or cirrhosis were completed (413).

Table 0-1: All measures of muscle mass, strength and functions evaluated

Muscle domain	Assessment modality	Variable measures
Muscle Mass	Ultrasound	Vastus lateralis muscle thickness (cm)
	MRI	Quadricep Cone volume (cm^3)
		Quadricep Volume index (cm^3/m^2)
		L3 skeletal muscle index* (cm^3/m^2)
	Anthropometry	Mid arm muscle circumference (cm)
	Bio impedance analysis	Fat free mass (kg)
Muscle strength	Isokinetic dynamometry	Knee extensor peak torque (Nm) Knee extensor average power (watts)
	Anthropometry	Handgrip strength (kg) (dominant peak)
Muscle function	Composite tests	Short physical performance battery (total, gait, balance and chair stand score, chair stand time) Liver frailty index
	Accelerometry	Overall daily activity (mg) Total inactive time (mins)

Magnetic resonance imaging (MRI); 3rd lumbar vertebral skeletal muscle index (L3 SMI), handgrip strength (HGS); midarm muscle circumference (MAMC), short physical performance battery (SPPB), liver frailty index (LFI).

3.2.3 Statistical analysis

All data were analysed using GraphPad Prism software, version 9 (La Jolla, CA, USA). The data were evaluated for normality with D'Agostino and Pearson test. All normally distributed data are presented as mean ± standard deviation and as median and IQR when non-normally distributed. The differences between the groups were analysed using a variety of methods including unpaired two tailed t-tests for normally distributed data and Mann Whitney U test for non-parametric distributed data. Correlation analysis was completed via Pearson's correlation for normally distributed data and Spearman's correlation for non-normal data. The level of significance for all tests was set at $p<0.05$.

3.3 Results

3.3.1 Description of cohort

Of the 39 participants from the study, included in this analysis, the median age was 58.0 (50.0-62.0) years, with a 59.0% male predominance (male to female ratio 23:16 participants). The leading aetiology in this ESLD cohort was ArLD (48.7%), with immune mediated disease (PBC and PSC) at 33.3% and NAFLD at 12.8%, and other causes 5.1%.

With regards to disease severity, this cohort had a mean MELD score of 13.4±4.7, UKELD 52.8±3.1 and Child Pugh score of 7.7±1.7 (class B). The mean bilirubin was 42.3±29.5 mmol/L, mean creatinine was 80.1±31.9 μmol/L , mean sodium was 137.1±3.6 mmol/L, mean INR of 1.3±0.3 and mean albumin of 35.1±5.9g/L. Full demographic data of cohorts can be found Table 3.2. In terms of complications of ESLD, 53.8 % had hepatic encephalopathy, all compliant with Rifaximin treatment. Portal hypertension was established in 90.1% of the cohort. with 73.8 % who had ascites. Of those who had ascites, 57.1% were diuretic controlled and 21.4 % were diuretic refractory undergoing frequent large volume paracentesis. Varices were confirmed in 59.5% with the majority (72.0%) treated with primary prophylaxis (beta blockage) .The most frequent medical co-morbidity was diabetes mellitus (11/39), of which 8 were insulin dependent.

The median length of follow up, from visit 2 until the end of the study (30th June 2021), was 1119 (1052-1207) days. By the end of the study, 16 patients (41.0%) underwent liver transplantation , 4 (10.2%) patients remained on the LT waiting list and 4 patients had died (10.2%)

Table 0-2: Baseline demographics of the End Stage Liver Disease (ESLD) cohort

N=39

Disease Severity	**Mean [S.D]**
MELD	13.6±4.7
UKELD	52.8±3.2
Childs Pugh score	7.6±1.7
Complications of ESLD	**Absolute value (%)**
Hepatic encephalopathy	21 (53.8%)
Portal hypertension	35 (89.7%)
Ascites	29 (74.4%)
Diuretics	23 (59.0%)
Large volume paracentesis	7 (17.9%)
Spontaneous bacterial peritonitis	9 (23.1%)
Varices	23(59.0%)
Primary prophylaxis	15 (38.5%)
Portal vein thrombosis	5 (12.8%)
Comorbidities	**Absolute value (%)**
Cardiovascular disease	4 (10.2%)
Hypertension	12 (30.8%)
COPD	6 (15.4%)
Diabetes mellitus	11 (28.2%)
Insulin dependent	*8 (20.5%)*
Hypercholesterolaemia	6 (15.4%)
Chronic kidney disease	7 (17.9%)
Outcome	**Absolute value (%)**
LT during the study	16 (41.0%)
On LT waiting list	4 (10.2%)
Deceased	4 (10.2%)
Biochemical parameters	**Mean [S.D]**
Haemoglobulin (g/L)	127.0±16.3
White cell count (10^9/L)	4.9±1.8
Neutrophils (10^9/L)	2.9±1.3
Platelets (10^9/L)	114.7±68.8
INR	1.3±0.4
Bilirubin (μmol/L)	43.5±29.5
ALT (IU/L)	53.3±58.9
ALP (IU/L)	252.5±295.4
Albumin (g/L)	35.1±5.9
Sodium (mmol/L)	137.1±3.6
Urea (mmol/L)	7.2±5.2
Creatinine (μmol/L)	80.1±32.0
EGFR	78.5±15.4
Ammonia (mg/ml)	73.8±40.0
HbA1c (mmol/mol)	38.5±16.0
CRP (mg/L)	7.3±7.3

3.3.2 Muscle mass

Thirty-nine participants (23 male/16 female) underwent an MRI of the lower limbs, ultrasound, anthropometry and BIA assessment. However, MRI measures of L3 SMI were only possible in 32/39 participants due to a technical error during the acquisition of the MRI images.

Quadriceps volume, peak quadriceps ACSA, L3 SMI and quadriceps volume index all demonstrated a significant correlation with each other (Figure 3.2). The strongest correlation was demonstrated between quadriceps volume index and quadriceps volume ($r = 0.94$, $p<0.0001$) and between quadriceps volume and peak quadriceps CSA ($r = 0.95$, $p<0.0001$) respectively.

Quadriceps volume (peak quad ACSA not vol) was the only measure which significantly correlated to all other measures of muscle mass (Figure 3.3). VL muscle thickness as assessed via ultrasound, was also found to correlate to all MRI measures of muscle mass measures including peak quadricep ACSA ($r = 0.57$, $p<0.0001$), L3 SMI ($r = 0.42$, $p <0.05$) and quadriceps volume index ($r = 0.57$, $p<0.001$) (figure 3.2 and figure 3.4). L3 SMI demonstrated a positive correlation with quadriceps volume ($r = 0.69$, $p<0.0001$), peak quadricep ACSA ($r = 0.65$, $p <0.001$) and quadricep volume index ($r = 0.68$, $p<0.0001$). Correlations of measures of muscle mass are shown in Figure 3.2 and 3.3, however, L3 SMI also correlated to ultrasound VL muscle thickness ($r = 0.42$, $p<0.05$).

MAMC was the only upper limb measure of muscle mass. It demonstrated some correlation to peak quadricep ACSA on MRI ($r = 0.34$, $p<0.05$). Finally, fat free mass as measured by BIA, correlated to all measures of mass on MRI and US, excluding MAMC (Figure 3.2).

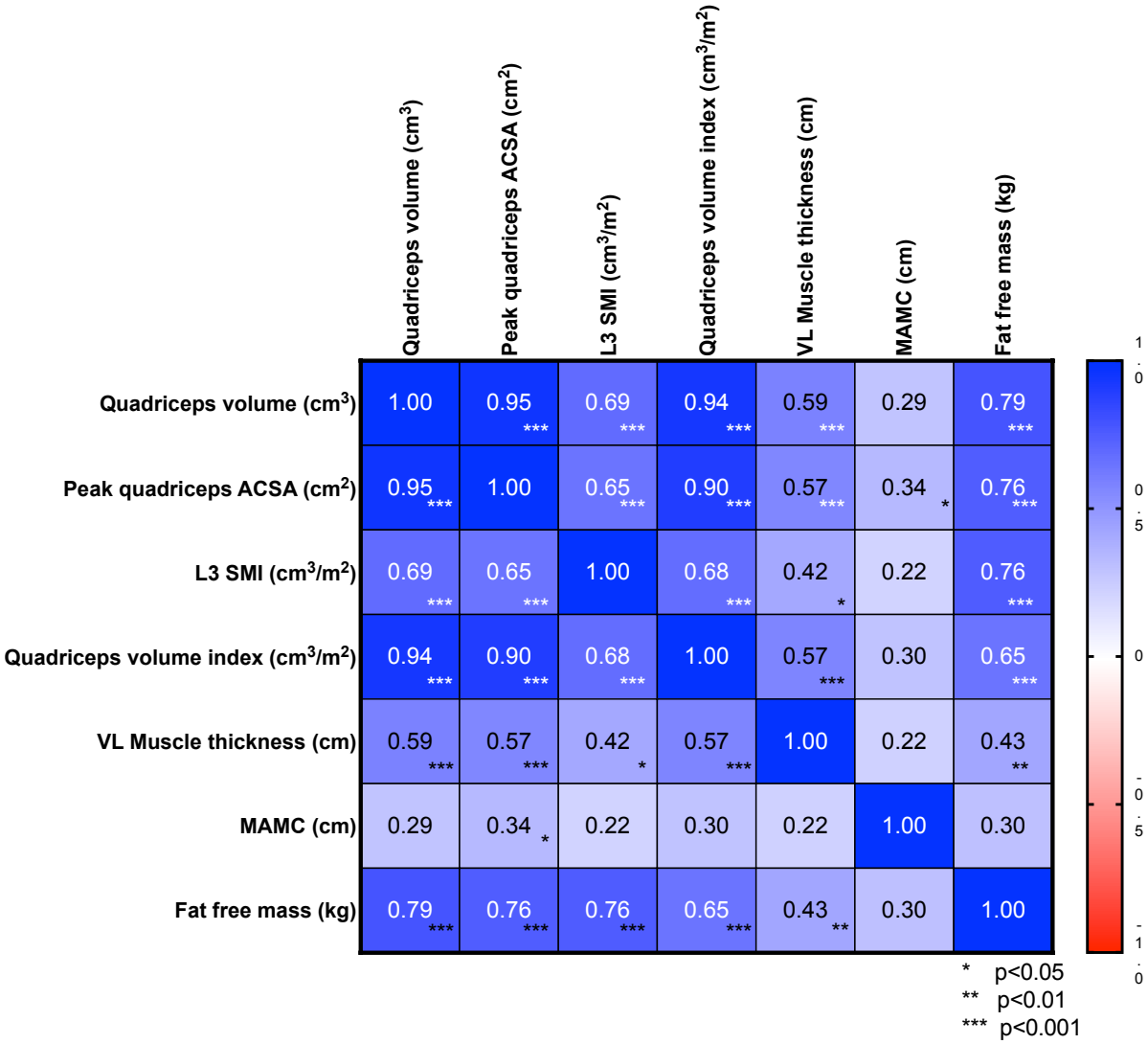


Figure 3-2: Correlation matrix for all measures of muscle mass

p values denoted by *p<0.05, ** p<0.01, ***p<0.001) via Pearson correlation.

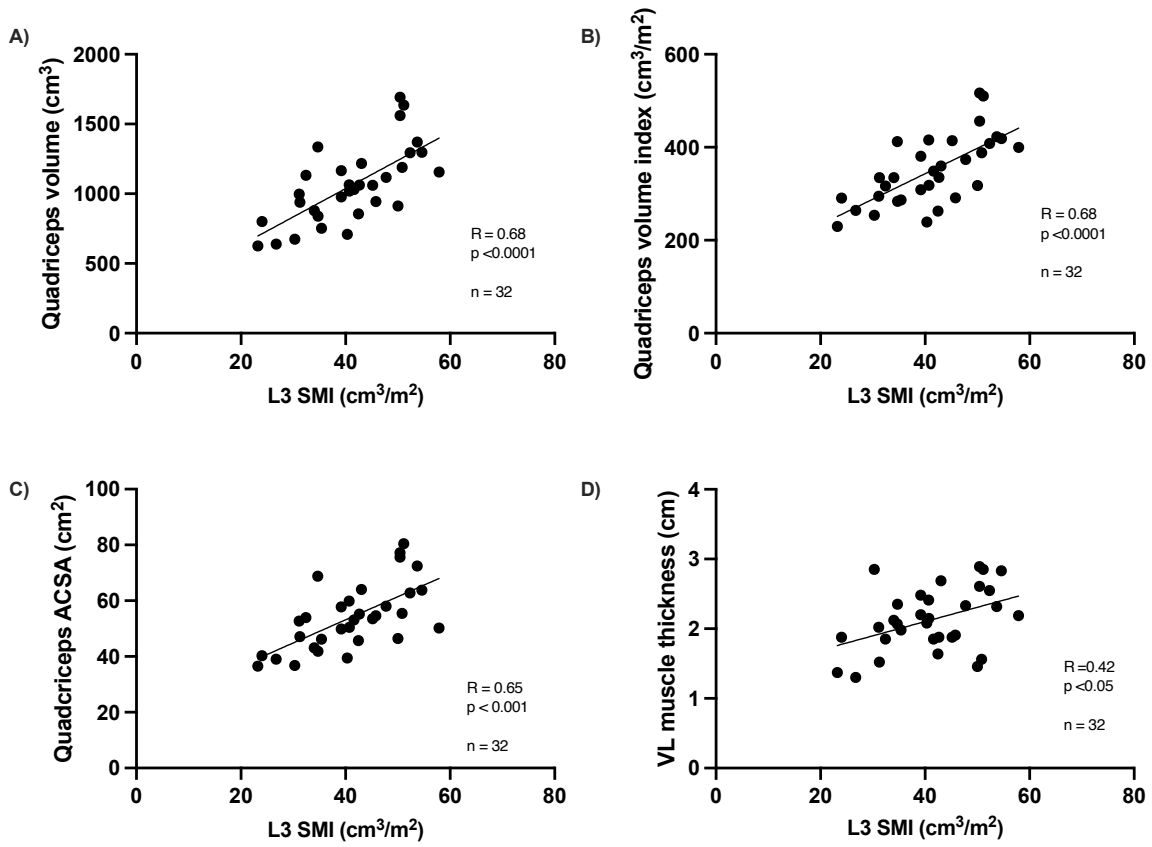


Figure 3-3: Correlation of L3 SMI to quadricep measures of muscle mass

Relationship between L3 SMI and MRI and US quadricep mass measures via linear regression analysis. A) Shows the correlation between L3 SMI and quadriceps volume, B) shows the correlation between quadriceps volume index and L3 SMI, C) shows the correlation for quadriceps peak ACSA and D) shows the correlation between VL muscle thickness and L3 SMI. P values as denoted for each figure.

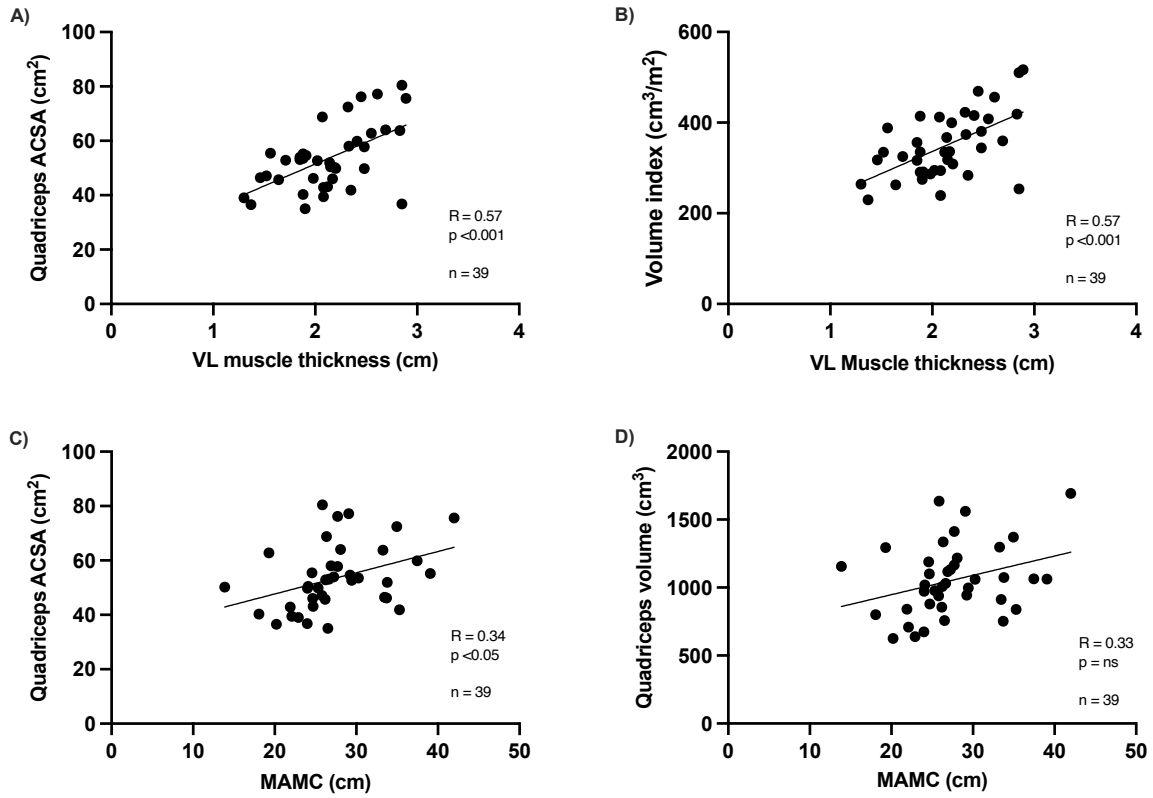


Figure 3-4: Relationship of measures of muscle mass

A) Shows the correlation between quadriceps peak ACSA and VL muscle thickness B) shows the correlation between quadriceps volume index and VL muscle thickness, C) shows the correlation for quadriceps peak ACSA and MAMC D) shows the correlation between quadriceps volume and MAMC. p values as denoted for each figure.

3.3.3 Muscle strength and function

Thirty-nine participants underwent various strength assessments for the upper (HGS) and lower limbs (maximal isokinetic knee extension), as well as composite performance tests (SPPB and LFI). Physical activity data, as assessed by wrist-worn accelerometers was also obtained.

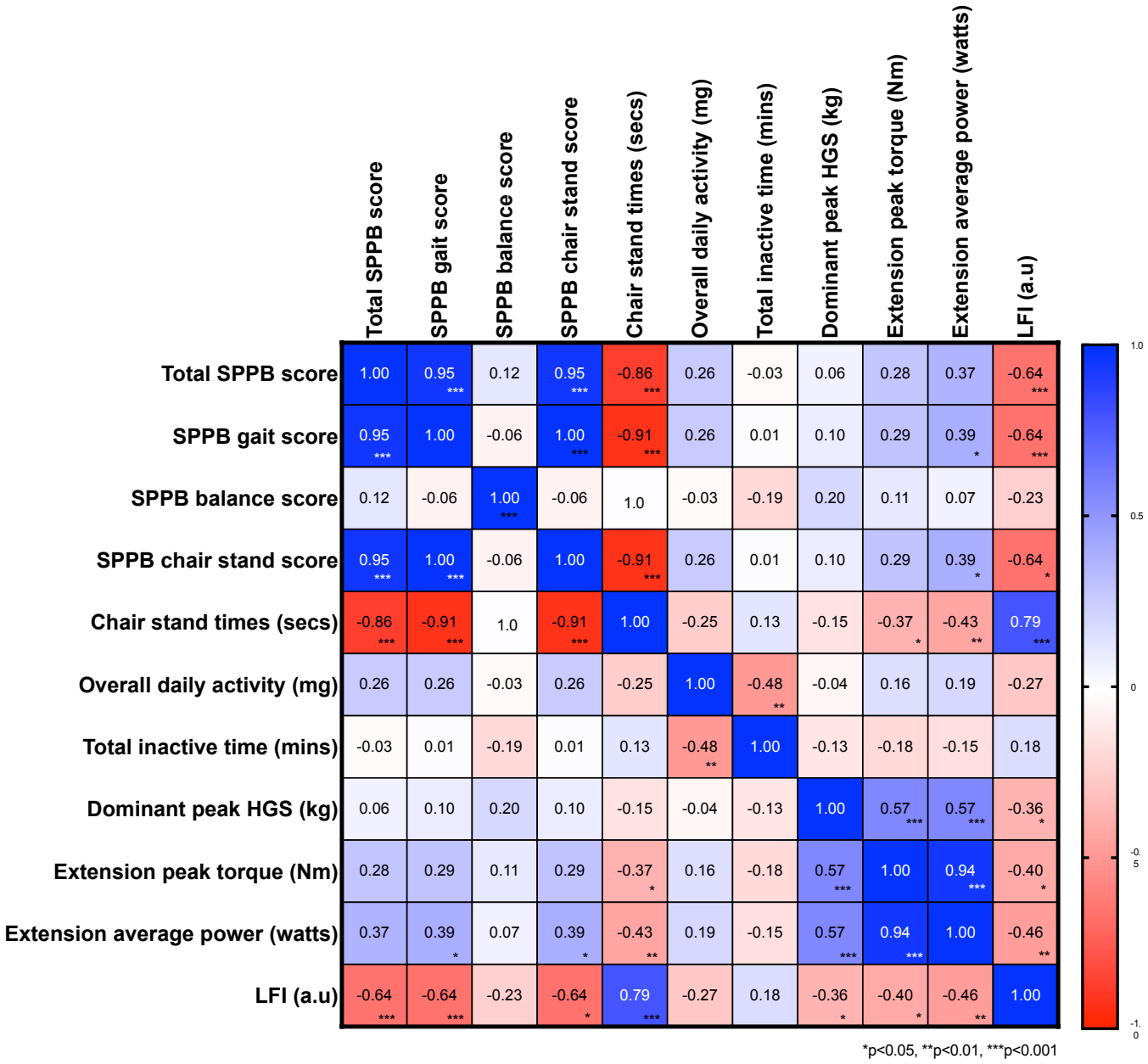


Figure 3-5: Correlation matrix for all measures of muscle strength and function

p values denoted by *p<0.05, ** p<0.01, ***p<0.001

On comparison of upper limb to lower limb strength, the results demonstrate significant correlation of peak dominant HGS to knee extensor peak torque (r = 0.57 p<0.0001) and knee extensor average power (r = 0.57 p<0.0001) (Figure 3.5 and Figure 3.6).

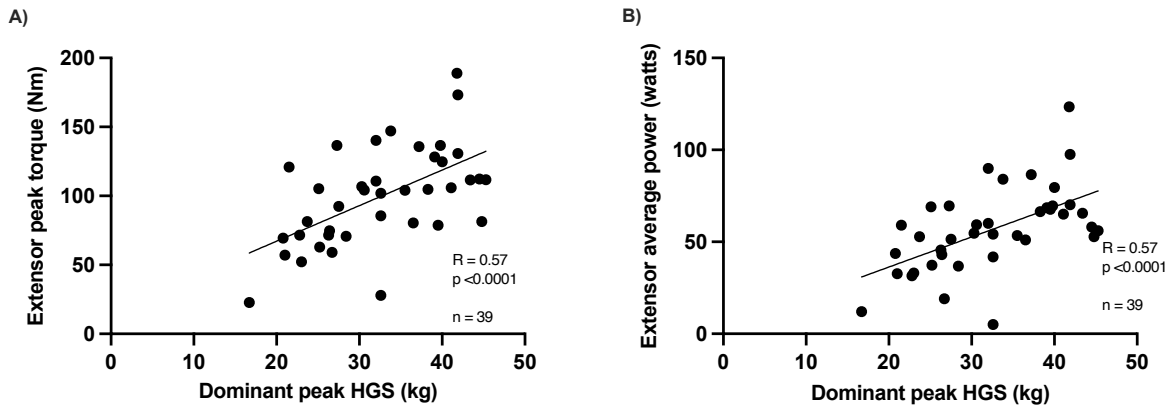


Figure 0-6: Relationship between upper and lower limb strength

This describes the relationship between upper and lower limb strength via linear regression analysis. A) Shows the correlation between peak dominant HGS and extensor peak torque B) shows the correlation between peak dominant HGS and extensor average power. p values as denoted for each figure.

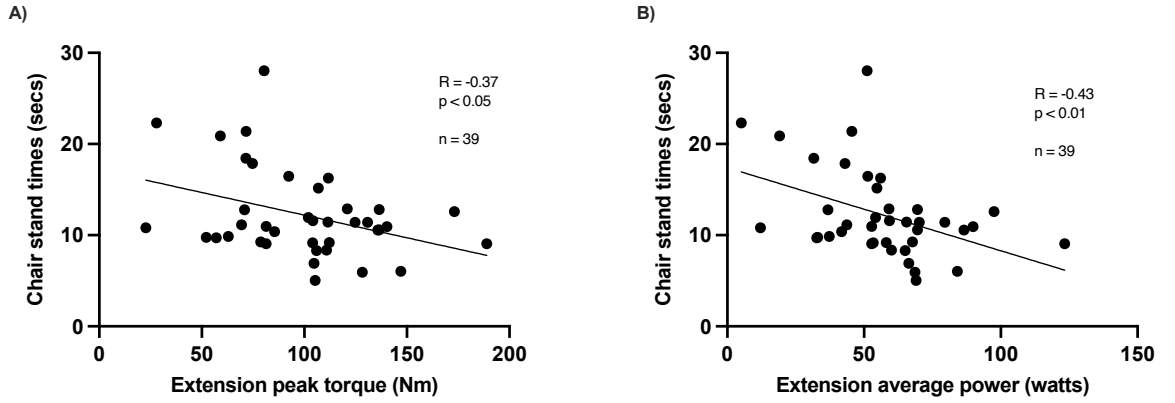


Figure 3-7: Relationship between the lower limb strength and function

The relationship between the lower limb strength and function via linear regression analysis. A) Shows the correlation between chair stands times and extensor peak torque B) shows the correlation between chair stand times and extensor average power. p values as denoted for each figure.

Lower limb measures of strength and function correlated to each other (Figure 3.7). Chair stands times inversely correlated to both leg extensor peak torque and leg extensor average power ($r = -0.37$, $p<0.05$ and $r = -0.43$, $p<0.01$ respectively).

Additionally, strength measures, HGS and knee extensor peak torque correlate to composite performance and functional measures such as LFI ($r= -0.36$, $p<0.05$) and ($r= -0.40$ $p<0.01$) respectively. HGS and knee extensor peak torque correlations to total SPPB were not found to be significant (Figure 3.5).

Composite functional measures, total SPPB and LFI demonstrated correlation to each other ($r= -0.64$, $p<0.0001$) (Figure 3.5). The results did not show a significant correlation of overall daily activity or inactivity time to any measure of limb strength, muscle function or composite functional tests (Figure 3.5).

3.3.4 Correlation between mass and function including muscle quality

In addition to the correlations above, the measures of muscle mass and muscle function were analysed for correlations between each other (Figure 3.8).

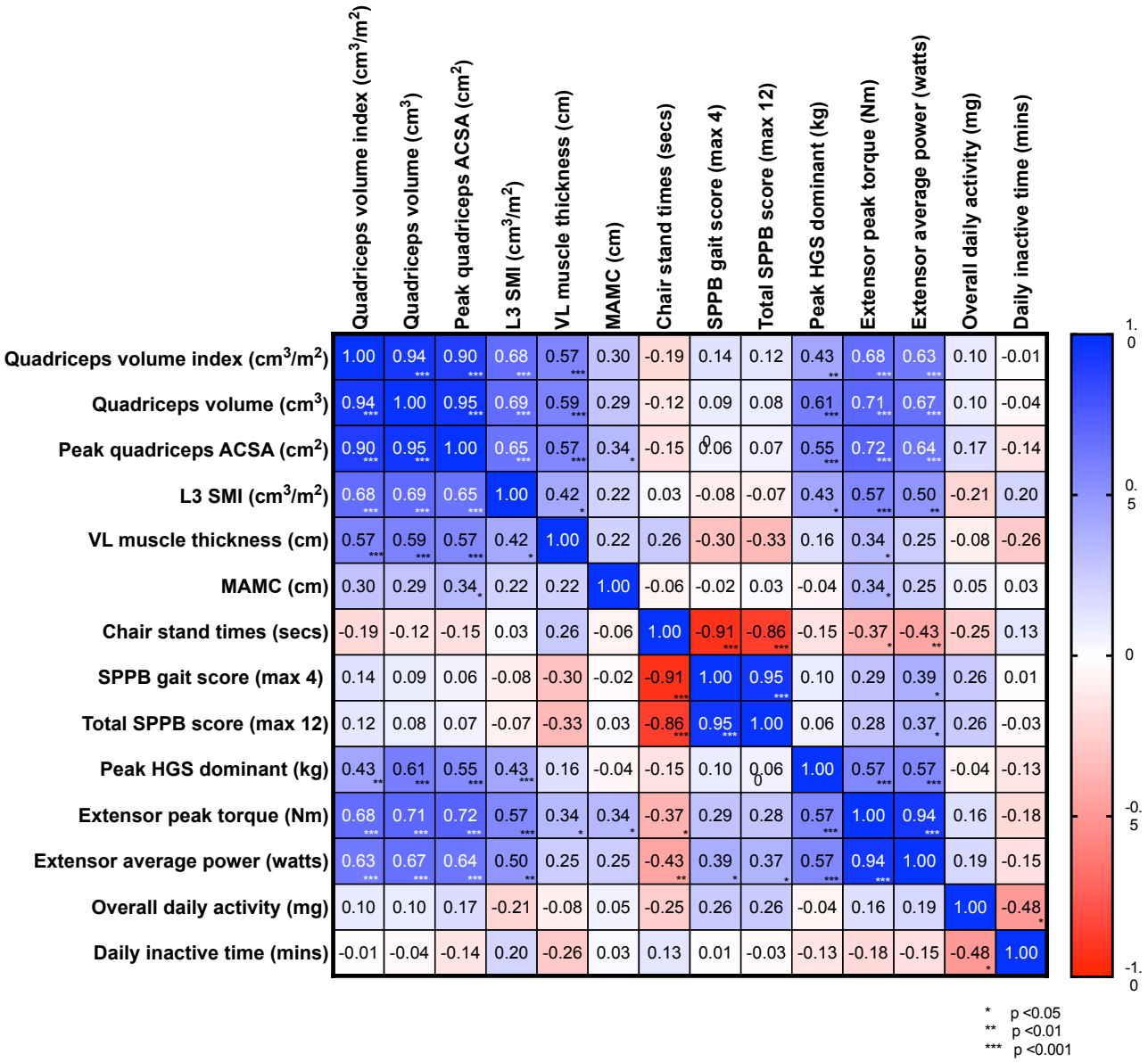


Figure 3-8: Correlation matrix for all measures of muscle strength and function

p values denoted by *p<0.05, ** p<0.01, ***p<0.001

All MRI muscle mass measures (quadriceps volume index, quadriceps volume, peak quadriceps ACSA and L3 SMI) correlated to all measures of performance (peak dominant HGS, knee extensor peak torque, and knee extensor average power). The strongest correlation of muscle mass to strength was demonstrated by MRI quadriceps volume to both upper limb strength (peak dominant HGS r= 0.61, p<0.0001) and lower limb strength (knee extensor peak torque r= 0.71, p<0.0001 and knee extensor average power r= 0.67, p<0.0001) (Figure 3.9 and

3.10). Ultrasound VL muscle thickness only correlated to knee extensor peak torque (r= 0.34, p<0.05) (Figure 3.10). There was no measure of muscle mass that correlated significantly to daily activity or inactivity time (Figure 3.8).

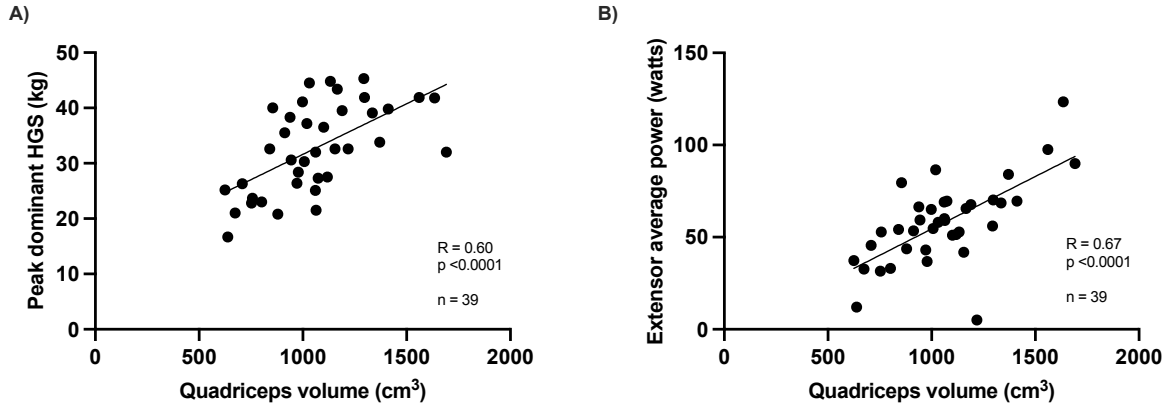


Figure 3-9: Relationship between upper and lower limb strength to quadriceps volume

The relationship between upper and lower limb strength to quadriceps volume via linear regression analysis. A) Shows the correlation between quadriceps volume and peak HGS B) shows the correlation between quadriceps volume and extensor average power. p values as denoted for each figure.

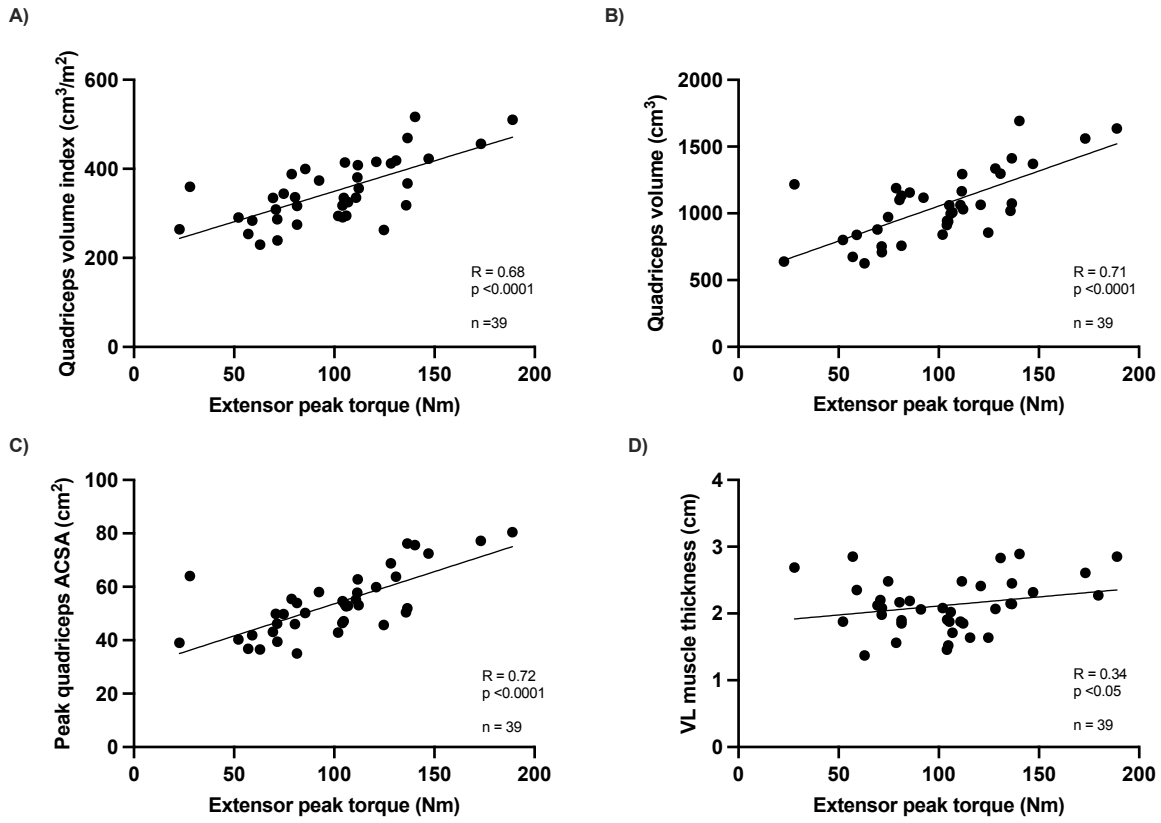


Figure 3-10: Relationship between the different measures of mass to lower limb strength

The relationship between the different measures of mass to lower limb strength via linear regression analysis. A) shows the correlation between quadriceps volume index and extensor peak torque, B) shows the correlation between quadriceps volume and extensor peak torque, C) shows the correlation between quadriceps peak ACSA and extensor peak torque D) shows the correlation for VL muscle thickness and extensor peak torque. p values as denoted for each figure.

Correlations between both upper limb and lower limb mass and strength measures were analysed (figure 10). All lower limb mass measures correlated to lower limb strength in the form of knee extensor peak torque (quadriceps volume r= 0.71, p<0.0001, quadricep volume index r= 0.67, p<0.0001, peak quadriceps ACSA r= 0.72, p<0.0001, and VL muscle thickness r= 0.34, p<0.05) (Figure 3.10). All lower limb MRI mass measurements correlated to knee extensor average power (Figure 3.10). Neither upper nor lower limb measures of muscle mass correlated to chair stands or gait (Figure 3.8).

3.4 Discussion

As there is no single consensus of the best measure of muscle mass or function in patients with ESLD, one main purpose of this study was to evaluate the degree of correlation between these variables of muscle mass, strength and function. This study, which I designed and led, therefore evaluated multi modal assessments of muscle mass, strength and function including measures of the trunk, upper and lower limb.

The current most frequently used standard method of measuring muscle mass in those with ESLD is L3 SMI (218, 219). it is shown here that muscle mass measures from the quadriceps (via MRI and US) correlate well to the existing measure of L3 SMI on MRI, suggesting that quadricep measures from MRI and US could be an alternative mode of measuring muscle mass, with less radiation than conventional CT obtained L3 SMI (219, 222) . The data also demonstrates that MRI quadriceps volume and peak quadricep ACSA possess the strongest correlation to other mass measures, including measures of lower limb mass and those more commonly used, such as L3 SMI and MAMC. In addition to the data described above, these results, again support the potential use of quadriceps muscle group in assessing muscle mass. It is well established that the quadriceps muscle group is highly susceptible to the earliest changes of muscle loss in primary sarcopenia (436-439) therefore it is plausible that a similar phenomenon could be validated in those with ESLD and muscle wasting. There has been one study to date by Tandon *et al* (222) utilising ultrasound by measuring muscle thickness, supporting its potential use; however further research is required to validate the used of quadriceps imaging in an ESLD cohort of 159 patients. In contrast, the upper limb mass measures of MAMC did not demonstrate a significant correlation to other mass measures, which has been demonstrated in previous studies (440, 441). This suggests that MAMC may

not be the most accurate method of measuring muscle mass, although one of the most commonly utilised in those with ESLD. Measures of MAMC can be highly operator dependent with inter-operator variability and reliability issues, however we mitigated for this by limiting the number of operators to three members of the research team and by taking an average of two measures.

The quadriceps represent a large muscle group, with a significant muscle volume as compared to upper limbs. There is a suggestion that smaller upper body muscle groups may demonstrate preserved muscle mass, as compared to larger lower body muscle groups as the latter is more involved in locomotion and therefore may be more susceptible to disuse (59, 436). This phenomenon has been observed in study of a general adult population with sarcopenia by Abe *et al* (comparing US measures of SMI to limb site specific measures of muscle thickness) to observe the detection of reduced muscle mass (442). Other possibilities for a greater loss of muscle mass in the lower limbs include the greater loss of motor units which has been hypothesised from indirect estimates via electrophysiological methods of measuring motor units in skeletal muscle (443), which further support the concept that larger muscle groups such as the quadriceps demonstrate reductions in muscle mass measure earlier than trunk of upper limb. This has yet to be explored in those with ESLD, but should be an area for future research.

Aside from limb specific differences, one of the aims of this study was to investigate the accuracy of different imaging modalities (i.e. MRI and US) at the same site, particularly within the lower limbs. The data show that there is correlation between vastus lateralis (VL) muscle thickness, as assessed via ultrasound, to other MRI quadriceps measures of mass. US

measures of muscle mass are gaining interest outside of those with ESLD. Studies in elderly populations have started to utilise this. A study in an elderly cohort of hospitalised participants found rectus femoris muscle thickness has a positive predictive value of 64.3% in identifying sarcopenia in accordance with EWGSOP criteria (444), with a more recent study in an similar elderly but outpatient cohort, demonstrating that rectus femoris muscle thickness has a AUC of 0.76 in predicting sarcopenia (diagnosed by HGS and BIA) (445). This supports the utilisation of ultrasound measures in measuring lower limb muscle mass. The benefits of ultrasound include bedside availability, no radiation exposure and easier accessibility. However, it is important to recognise that in this case, we only measured VL thickness and as such it does not take into consideration the other muscles within the quadriceps group as compared to the MRI quadriceps measures of mass. VL consists of ~33% of the quadriceps muscle group (446), this may suggest that the correlation of VL muscle thickness to other MRI quadriceps measures may be stronger by using other ultrasound measures of muscle mass such as the extended field of view measure (described in Chapter 2) which incorporates all of the quadriceps muscle.

With regards to muscle function, the study results showed that upper limb strength via HGS, correlated to lower limb strength measures including knee extensor peak torque. It is worth nothing that peak dominant HGS was used from this study visit to compare to other measures, the data do show a strong correlation of dominant to non-dominant HGS ($r=0.95$, $p<0.0001$). Furthermore, peak torque of the dominant limb was compared to the non-dominant limb from the baseline data for Visit 1 which also showed a strong correlation between the two measures ($r=0.89$, $p<0.0001$). As such, I do not feel the use of dominant or non-dominant limb strength has impacted the results. As HGS is the most frequently used measure of muscle

strength (197, 227), however this supports the potential use of knee extensor peak torque as a strength measure when HGS is inappropriate, insufficient or contraindicated. In addition, sole reliance on an assessment of upper limb strength may result in the patient having an inaccurate surgical risk assessment or intervention evaluation (i.e. nutrition), when lower limb strength/function is so important for overall functional independence.

Unfortunately, we did not see any demonstrable relationship between strength measures (HGS and leg extensor peak torque) with any of the individual or total SPPB scores. This may be due to the ceiling effect from the maximal scoring that occurs within the components of the SPPB. Indeed, the separation of the scores and the use of chair stand times, demonstrated correlation to leg extensor peak torque. The quadriceps are a principal muscle group involved in performing chair stands, whereas gait and balance involve several other large muscle groups, proprioception and vestibular and visual integrity. Chair stand times may have demonstrated a correlation given the large involvement of the quadriceps with the chair stand times rather than the equivalent score overcoming the ceiling effect of the test. Furthermore, some correlations with performance measures may not show a linear relationship, therefore may not be evident within the single time point used within this study.

Measures of daily physical activity and inactive time did not show a correlation to other mass or functional measures during this analysis. Physical activity has a key role in the preservation of muscle mass and strength (134) with reduced physical activity driving muscle wasting (276) and so this result was unexpected. Physical activity assessment via accelerometry has rarely been performed accurately in those with ESLD, with the exception of step count as the measured variable, thus there is a paucity of data for comparison. We may not have

demonstrated a correlation in this analysis, possibly due to the small number of accelerometry data sets (n=30). Those with more than 4 days' worth of data were included. Importantly, the control cohort was recruited from the University of Birmingham and University Hospitals of Birmingham following the Sars-CoV2 pandemic. There was a work from home mandate during this time, and this may have impacted the control data and the analysis as a result. We used a two-week time period to collect the accelerometry data to try and ensure an adequate representation of daily activity measures. Therefore, for the purpose of this analysis, only selected broad variables observing mean intensity of movement have been selected. These variables may not be sufficient to demonstrate the changes between other measures of muscle function. There are no studies to date that evaluate physical activity (excluding subjective questionnaires or arbitrary step counts), which highlight the unique aspect of this study in measuring physical activity in this cohort. Furthermore, this suggest that physical activity requires further exploration as inactivity strongly correlates with the development of both primary and secondary sarcopenia (134). Within the accelerometery data itself, there are many variables including intensity gradient of activity, level of activity e.g. moderate, light, strenuous that may demonstrate correlations which requires an in-depth complex analysis, not within the remit of this thesis. Other important considerations that may also have a potential impact in assessment of physical activity include motivation, quality of life and behaviour. Again, these are not included within the remit of this thesis.

The cross comparison of the measures of muscle mass and function showed that all MRI quadriceps mass measures and L3 SMI had a significant correlation to upper and lower limb strength measures, in contrast to MAMC and VL muscle thickness. Further correlation of muscle mass and function measures demonstrated the lower limb mass and function

measures correlated to each other more than upper limb measures of mass and function. Whilst it can be appreciated that knee extensor strength via peak torque and MRI and US quadriceps measures the same muscle group, the same is not true for the upper limb measures. MAMC measures biceps and triceps muscles whereas HGS utilised a greater proportion of forearm strength. It does support the consideration of using limb specific mass and function measures to further phenotype those with ESLD for sarcopenia and frailty.

Function and strength measures can be affected by clinical features such as ascites, peripheral oedema and hepatic encephalopathy. This is noticeable in BIA and DXA where the ascites and peripheral oedema interfere with the accurate interpretation of fat free mass as a surrogate of lean muscle mass. Adaptations to optimise these should be a consideration when assessing muscle strength or function in ESLD patients (219). Lower limb methods of measuring muscle mass are less susceptible to interference from ascites and complications of ESLD. Additionally, lower limb muscle mass has been related to overall function and independence in non-ESLD cohorts (435), and provides a larger muscle group for glucose metabolism and muscle homeostasis. Hepatic encephalopathy, a commonly complication of ESLD, remains a challenge in measuring muscle function. Overt HE has a clear negative impact on compliance and understanding consequently may impact the ability to perform functional assessments to a true maximum. However covert HE may manifest in subtle underperformance of measures of muscle strength and function. Nonetheless, optimisation and consideration of HE remains essential when completing muscle function assessments.

This chapter has focussed on measures of muscle mass (quantity) and performance (strength and function). An important consideration of muscle function includes the contractility of the

muscle tissue, which in turn contributes to the ability of the muscle to generate force. This can be impacted by several factors including the integrity and function of the neuromuscular junction, the architecture of the muscle (fascicle length and pennation angle) and intramuscular and intermyocellular adipose deposition within the muscle (447). The various methodologies to evaluate these features will be explored in chapter 4.

3.5 Conclusion

In conclusion, the data demonstrates that there is correlation between the commonly used standard measure (L3 SMI) to MRI and US measures of quadriceps) muscle mass. Further, lower limb mass measures of quadriceps from MRI correlate to other measures of upper and lower limb strength and function. This provides support for the use of lower limb measures of mass in ESLD and suggests that there should be further consideration of evaluating functionally relevant muscle groups such as the quadriceps to identify those with sarcopenia and/or frailty. I also found that measures of upper and lower limb strength (HGS and leg extensor peak torque) correlate significantly with each other, therefore this may be used in replacement of each other when required by clinical circumstances. Composite measures of function such as the LFI, also demonstrated significant correlation to measures of HGS and chairs stands time used within it, and other measures of strength (leg extensor peak torque) and SPPB.

Overall this study presents an in-depth correlation analysis of the measures of muscle mass, strength and performance used in ESLD, which has not been done elsewhere to date, and suggests consideration of these measures are required when implementing them to phenotype sarcopenia or frailty in patients with ESLD.

4. Chapter Four: Phenotype of muscle mass and function in End Stage Liver Disease and the impact of disease aetiology, severity, age and sex

4.1 Introduction

The impact of muscle wasting in End Stage Liver Disease (ESLD) has gained increasing exposure in the last decade. Within the remit of liver disease, sarcopenia is defined as the loss of muscle mass as defined by L3 SMI, with the concept of compromised muscle function is incorporated in the terminology of physical frailty (256). The current prevalence of sarcopenia in cirrhosis ranges from 30-70% (71, 222) however whether these estimates would change with the inclusion of muscle function measures remains largely unexplored. The mechanisms driving muscle wasting in cirrhosis are complex and multifactorial. The imbalance between protein catabolism and synthesis likely occurs due to reduced protein intake and synthesis, increased muscle catabolism and increased energy expenditure (197, 227). This is described in detail in Chapter 1. There are factors specific to those with ESLD which play a role in muscle wasting and need consideration. For example, hepatic encephalopathy can result in reduced physical inactivity as a direct consequence of cognitive dysfunction and hyperammonaemia in turn can also inhibit protein synthesis and muscle regeneration by upregulating myostatin (205, 281).

Previously, Chapter 3 discussed the individual modalities for assessing muscle strength and function in patients with ESLD. Herein, the aim of this chapter is to assess the impact of disease aetiology and severity, in addition to age and sex, has upon muscle mass and function in patients with ESLD.

ESLD disease severity : Muscle wasting is partially influenced by the severity of liver disease, this is in part because progressive liver failure itself is a driver of sarcopenia. There is a greater prevalence of sarcopenia (defined as muscle mass loss) through the increasing stages of disease severity defined by Child Pugh scoring from a recent meta-analysis (448). The prevalence of sarcopenia in those with Child Pugh C was 46.7%, (95% CI 39.0%-54.5%,) compared to 37.9% in those with Child Pugh class B (95% CI 29.9%- 46.3%) and 28.3% in Child Pugh A (95% CI 20.5%-36.8%) cirrhosis (448). Established scoring systems for liver disease such as MELD do not include muscle mass or functional parameters in their scoring algorithms. Both MELD (minus sodium) and UKELD utilise serum sodium, bilirubin, creatinine and INR to calculate liver disease severity (449, 450). Montano Loza *et al* devised a scoring system, named MELD-sarcopenia, which incorporates gender specific L3 SMI cut-offs, to evaluate the prediction of mortality when incorporating muscle mass (451). The MELD-sarcopenia score outperformed MELD at predicating overall, 3, 6, and 12 month mortality, however only with an observed significance in those with a MELD <15 (i.e. less severe disease) (211). This was validated by Van Vugt *et al* who found a similar discriminative performance of the MELD-sarcopenia for 3-month mortality in their European cohort (c-index 0.82) (452). Other scoring systems which have incorporated sarcopenia into disease severity scoring included in MELD psoas score (453). These studies only measured psoas muscle area using CT, as opposed to cross sectional muscle areas which has been previously validated. It produced conflicting results and overall psoas muscle measurements are less well validated, suggesting it is an inferior measure in ESLD (325, 453).

Complications of ESLD, such as hepatic encephalopathy (HE) and refractory ascites, evolve as the disease severity worsens. The role of HE is important in the development of sarcopenia

as there is an important association between those with reduced muscle mass and HE (454). In cirrhosis, the damaged liver is unable to effectively remove ammonia through the normal cycle of urea synthesis. Skeletal muscle compensates this by increasing its ammonia uptake (205). Skeletal muscle consequently removes ammonia by converting it into glutamine via contains glutamine synthetase(205). However, this process also results in a loss of alpha ketoglutarate (a key molecule in the Krebs cycle), leading to increased cataplerosis and hence mitochondrial dysfunction, which results in the loss of ATP substrates; collectively this can result in dysregulated protein synthesis, as described in Chapter 1. Thus, the loss of muscle mass, further impairs ammonia clearance, in addition to the existent impaired clearance from the primary hepatocellular dysfunction, and consequently accelerates HE (238, 455, 456) . In addition, the resultant hyperammonaemia from HE, drives muscle catabolism via upregulation of myostatin and mitochondrial dysfunction, worsening the loss of muscle mass (455, 457). HE has a negative impact on physical function due to psychiatric, motor and cognitive factors which with increased somnolence, impaired memory and fatigue; all which impair nutritional intake, compliance with treatment, and physical function and activity (458).

Aside from HE, ascites is a common complication of advanced portal hypertension. It is reported that those with significant ascites have difficulties in performing simple activities of daily living (ADLs), including walking and climbing stairs, not to mention more formal exercises (running, swimming, resistance weights), resulting in overall reduced daily physical functioning (220). Furthermore, ascites can cause reduced appetite and reduced dietary intake including protein, further promoting further sarcopenia and physical deconditioning (459). The accumulation of this usually protein containing fluid within the peritoneal cavity, adds additional weight; this fluid falsely implies greater BMI and the excess fluids may impair

muscle mass and functional measurements(197, 459). Additionally, the sequestration of protein rich fluid into the abdominal space is a contributing factor in protein loss (460). It is therefore, unsurprising that there is a strong bi-directional relationship between the presence of ascites and sarcopenia. Indeed, Topan *et al* performed a prospective study that identified that sarcopenia (defined as muscle mass by L3 SMI and dominant HGS) increased the risk of the development of ascites by 3.78 fold (461), again emphasising the importance of managing muscle wasting in those with ESLD.

Aetiology of ESLD : The aetiology of liver disease may have an impact on the degree of muscle wasting in those with ESLD. For example, in those with alcohol related liver disease (ArLD), the excessive consumption of ethanol results in the dysregulation of muscle proteolysis via negatively impacting mTOR pathway with signalling perturbations, accelerating proteolysis, and subsequently producing a more severe sarcopenic phenotype (462). Whilst the cessation of alcohol is integral for reducing the rate of liver disease progression and decompensating events, the muscle wasting appears to persists beyond abstinence (245). Those with an alcohol misuse disorder, often have a reduced yet imbalanced calorie intake with a larger proportion of calories from alcohol itself. Moreover, excessive consumption of alcohol, can suppress appetite and can lead to micronutrient deficiencies, malnutrition and results in muscle loss (245, 318). In contrast to ArLD, the physical appearance of muscle mass in those with NAFLD may appear less severe due to an increased degree of myosteatosis, which masks muscle mass loss by preserving muscle quantity on some common used measures such as MAMC, yet can affect muscle function and frailty disproportionately compared to relative muscle mass loss (463, 464). The concurrent sarcopenic obesity that is frequently seen in those with NAFLD will also influence these changes of increased myosteatosis, and preserved

muscle quantity (465). The additional drivers for sarcopenia in NAFLD include increased insulin resistance, decreased adipokinin and increased oxidation stress from the excess adipose (215, 464, 466). Further studies are needed to compare the drivers and phenotypes of sarcopenia for each disease aetiology.

Age: Advancing age itself, can enhance vulnerability to acute liver injuries and is associated with the severity of liver disease and an overall poorer prognosis (467). It has been well established that ageing plays a significant role in diminishing muscle mass and strength in the general population. With increasing age, muscle mass strength declines at a faster rate than the loss of muscle mass, in a non-linear fashion(468). After the 5th decade of life, there is a loss of 0.5-1% of muscle mass annually, whereas there is a greater loss of muscle strength (1.5% annually)(469, 470). Age related loss of muscle mass and function are also compounded by other determinants such as sex and ethnicity. Age related, or primary sarcopenia is driven by several influences including cellular senescence, alteration of metabolic pathways including mitochondrial dysfunction and oxidative stress, anabolic resistance, hormonal variation and a decline in physical activity (238, 467). There is also the conversion of increase of type 2 muscle fibres into type 1 fibres due to the loss of motor units, resulting in reduced force output. Finally there is an increased fat deposition, i.e. myosteatosis can, which impedes contractility and reduced the function of the muscle(238).

Sex: It has been well established that there are differences in body composition between the sexes since birth, however this becomes more prominent following the hormonal influences during adolescence. Females have increases in fat mass whereas males increase in lean mass which follows through to adulthood where women usually have a lower lean mass percentage

(238, 471, 472). Further, the distribution of muscle mass differ, with men have an increased distribution of upper and lower body muscle than women until the 5th decade. There is then a greater decline in lower limb muscle mass from the fourth to ninth decade loss in men than women (approximately 50% loss in men and 30% in women as measured by ultrasound quadricep thickness) (238, 471, 472). This alterations result in similar lower body muscle mass deposition in both sexes after the 8th decade (472). Thus, for primary sarcopenia, there are different sex cut-offs for both measures of muscle mass (appendicular skeletal mass), strength (HGS) and performance (chair stands, gait speeds, SPPB and timed get up and go tests) (59). It is important to note that there are recommended sex differences in muscle mass cut-offs in patients with liver disease however the functional sex specific cut-offs remain outstanding (256).

Therefore, the aims of this chapter are to compare the differences in measures of muscle mass, quality, strength and performance) in those with ESLD compared to an age, sex-matched healthy control cohort. Furthermore, the impact of the different aetiologies of ESLD, liver disease severity, age and sex on measures of muscle mass, quality and function, compared to the control cohort, will be investigated to explore any further consideration when evaluating those with sarcopenia

4.2 Methods

4.2.1 Study population

Chapter 2 outlines the study schedule including each visit, the time frame and the assessments measured at each visit. Fifty-three participants with ESLD were recruited to this study with 18 age sex matched healthy control participants. Of these 53 patients, only 42

patients with ESLD proceeded to their Visit 2 (Figure 4.1). Eleven participants did not attend their Visit 2 due to various factors, described in Chapter 3, with 5 patients withdrawing from participation in the study after their first visit (Figure 3.1)

For the assessment of fixed variables such as age, sex, aetiology and disease severity, the following grouping was selected (Table 4.1). For the age category, the age grouping, was based on the natural history of cirrhosis progression to allow an even spread between the groups. Disease severity was categorised by the UKELD scoring according to those being assessed for transplantation. Historically, a UKELD of 49 or greater indicates a survival advantage for LT over a conservative management approach in those with ESLD and is the minimal listing criteria for elective LT unless they have a variant syndrome (i.e. polycystic liver disease, recurrent cholangitis) or fulfil an alternative criteria (185, 473). Based on prognostic value, participants were divided into UKELD <50, 50-56 and >56, with the latter being the most severe and having the worst prognosis (473, 474).

Disease aetiology was categorised into the three main causes for liver disease, namely ArLD, immune mediated (which incorporated PSC and PBC only as there were no patients with AIH recruited) and NAFLD. There were 2 'other' patients with non-cirrhotic portal hypertension and cryptogenic cirrhosis which were excluded from the disease aetiology specific analysis. Some participants had a co-existing aetiology, however for the purposes for this study, the main aetiology (as proven by biopsy or laboratory testing) was used for this categorisation.

For the subgroup analysis of ascites, the ESLD patients were divided into those with diuretic refractory ascites, those with diuretic controlled ascites and those without any ascites. There

was one patient who had very mild ascites and stopped taking any medication during their participation within the study; this participant was therefore excluded from this subgroup analysis as their ascites category changed, hence n=41.

Table 4-1: Participant number for age, disease severity, aetiology and gender analyses

Age	20-49 years	50-59 years	60-71 years	Controls
N=42	10 (*9)	18 *(17)	14(*13)	8
Disease severity	UKELD ≤49	UKELD 50-56	UKELD >56	Controls
N=42	8	29*(26)	5	18
Aetiology	ArLD	Immune mediated	NAFLD	Controls
N=40	20 (*19)	14 (*13)	6 (*5)	18
Gender	ESLD Male	ESLD Female	Control Male	Control female
N=42	26 (*23)	16	11	7
HE	ESLD with HE	ESLD without HE	Control	
N=42	24 (*22)	18 (*17)	18	
Ascites	ESLD diuretic refractory ascites	ESLD diuretic controlled ascites	ESLD without ascites	Controls
N=41	9 (*8)	21	11 (*9	18

*Those within each group were MRI data was available

4.2.2 Assessment modalities and methods

Chapter 2 has a detailed description of all assessment modalities performed. Chapter 3 describes all the muscle mass, strength and function elements measured. The data included for mass and function variables was collected from Visit 2. Thirty nine of the 42 ESLD patients who attended Visit 2 underwent an MRI (due to technical and safety issues encountered at the time), therefore the MRI variables are based on n=39. Visit 1 and 2 took place approximately 14 days apart. Any missing data from this Visit 2 for blood results, height, and weight, were utilised from those taken at Visit 1. Accelerometry data were available for only 30 ESLD patients and 17 control participants.

4.2.3 Statistical analysis

All data were analysed using GraphPad Prism software, version 9 (La Jolla, CA, USA). The data were evaluated for normality with D'Agostino and Pearson test. All normally distributed data are presented as mean ± SEM, and as median and IQR when non-normally distributed.

The differences between the groups were analysed using several methods including unpaired two tailed t-tests and a one-way ANOVA for normally distributed data; and a Mann Whitney U test or Kruskal Wallis ANOVA for non-parametric distributed data. The level of significance for all tests was set at $p<0.05$.

4.3 Results

4.3.1 Baseline demographics of ESLD cohort

There were 42 participants with ESLD included in this study; the median age was 57.5 (IQR 50.0-61.3) years with 61.9% male predominance. Participants with ESLD were age and sex-matched to 18 healthy control participants, with a median age of 51.5 (IQR 33.0-63.3) years and a similar male predominance of 61.1%. The leading ethnicity of both cohorts was white Caucasian. The ESLD cohort had a median MELD score of 12.0 (10.8-15.3), median UKELD score of 52.5. (51.0-55.0) and a median Child Pugh score of 8.0 (6.0-9.0). The demographic details of the cohorts are shown in Table 4.2.

Table 4-2: Baseline demographics and biochemical parameters of the End Stage Liver Disease (ESLD) and healthy control cohorts.

	ESLD (TOTAL)	ARLD	IMMUNE MEDIATED	NAFLD	OTHER	CONTROLS
N (%)	42	20 (47.6)	14 (33.3)	6 (14.3)	2	18
Age (years)	54.6±10.6	56.4±5.7	53.4±16.1	50.0±8.2	59.5 [2.1]	50.0±15.2
Male	26 (61.9)	13 (65.0)	9 (64.2)	2 (33.3)	2	11 (61.1)
Female	16 (38.1)	7(35.0)	5 (35.7)	4 (66.7)	0	7 (38.9)
BMI (kg/m2)	29.9± 6.5	31.4±5.6	26.9±6.0	34.4±7.5	24.3±3.3	25.1±4.3
Dry BMI (kg/m2)	28.1±6.1	28.0±4.9	27.4±6.9	32.0±7.5	22.5±4.2	NA
BIOCHEMICAL PARAMETERS						
Haemoglobin (g/L)	130.0 [120.8-137.0]	129.0 [113.3-136.5]	131.0 [124.5-139.0]	126.0 [120.8-136.0]	132.5 [7.8]	134.0 [113.0-144.5]
White cell count (10^9/L)	4.3 [3.4-6.3]	4.0 [3.3-4.6]	6.1 [3.4-6.7]	4.9 [3.9-7.5]	3.7 [0.1]	4.9 [4.5-5.6]
Neutrophils (10^9/L)	2.6 [1.8-3.4]	2.5 [1.8-3.0]	3.4 [1.7-4.1]	3.1[1.7-5.0]	2.3 [0.4]	2.8 [2.2-3.1]
Platelets (10^9/L)	94.0 [64.0 – 127.8]	85.0 [64.5-113.5]	110.0 [80.0-236.0]	88.5 [62.5-114.3]	80.0 [36.8]	237.0 [204.5-265.8]
INR	1.2 [1.2-1.3]	1.3 [1.2-1.5]	1.2 [1.0-1.3]	1.3 [1.2-1.5]	1.3 [0.1]	1.0 [0.9-1.0]
Bilirubin (μmol/L)	34.5 [22.8-50.8]	35.0 [25.0-51.5]	41.0 [29.8-53.0]	23.5 [16.3-52.5]	21.5 [0.7]	12.0 [8.0-16.0]
ALT (IU/L)	29.5 [19.8-53.8]	21.0 [17.0-29.3]	80.0 [42.3-146.0]	26.5 [18.3-33.0]	49.5 [31.8]	18.0 [17.0-23.0]
ALP (IU/L)	168.0 [105.0-280.5]	138.0 [91.5-170.5]	375.5[236.8-517.3]	111.5 [80.8-183.0]	80.0 [36.8]	71.5 [58.3-76.5]
Albumin (g/L)	35.0 [30.8-40.0]	34.5 [30.3-37.8]	36.0 [30.1-41.0]	34.5 [28.8-43.0]	36.0 [1.4]	42.5 [40.0-45.3]
Sodium (mmol/L)	137.0 [135.0-140.0]	135.0 [134.0-139.5]	138.0 [137.0-141.0]	137.0 [134.0-140.3]	139.0 [2.8]	140.0 [138.8-141.0]
Urea (mmol/L)	5.8 [4.1-8.9]	6.4 [4.1-9.6]	5.2 [4.3-7.1]	8.0 [3.2-13.5]	8.6 [6.2]	5.1 [4.5 -5.7]
Creatinine (μmol/L	73.5 [58.0-88.8]	73.0 [57.3-94.5]	76.0 [57.5-85.7]	70.5 [62.3-98.8]	88.0 [26.9]	81.0 [68.0-88.0]
EGFR	86.5 [70.3-90.0]	90.0 [64.0-90.0]	84.5 [77.0-90.0]	80.0 [67.5-90.0]	62 [62.0]	83.5 [74.3-90.0]
Ammonia (mg/ml)	62.0 [51.7-76.3]	71.0 [56.0-89.8]	57.0 [41.0-82.5]	58.0 [41.5-129.0]	63.5 [2.1]	NA
HbA1c (mmol/mol)	33.0 [28.0-48.0]	33.5 [27.8-59.0]	31.5 [27.0-35.3]	51.0 [26.0-76.5]	36.0 [2.8]	34.5 [31.0-37.0]
CRP (mg/L)	5.0 [3.0-10.5]	6.0 [3.0-12.0]	6.0 [2.5-11.5]	5.0 [2.5-9.5]	1.0 [2.0]	1.0 [1.0-1.3]

*data presented a medians with [interquartile range], mean with ± standard deviation or as absolute value with (percentage)

The main aetiology of liver disease was ArLD (47.9%) followed by immune mediated disease (primary biliary cholangitis and primary sclerosing cholangitis exclusively; 33.3%) and NAFLD (14.3%). The ArLD subgroup had a greater disease severity with a median MELD of 14.0 (11-

17.8) median UKELD of 55.0 (52.3-55.8) and Child Pugh score of 9.0 (8.3-9.0) and a greater prevalence of complications of ESLD. All 20 participants with ArLD had hepatic encephalopathy (all compliant with Rifaximin treatment), portal hypertension and ascites. Of those with ascites, 70.0% were treated with diuretics and 35.0% treated with frequent large volume paracentesis. The immune mediated subgroup had the most deranged liver enzymes of all three subgroups with a medial bilirubin of 41.0 [29.8-53.0], median ALT 80.0 [42.3-146.0] or and median ALP 375.5[236.8-517.3]. The synthetic function as demonstrated by albumin and INR, was similar in all three subgroups of ArLD, immune mediated and NAFLD subgroups, respectively (Table 4.2).

In respect to outcomes, the median follow up time was 1119 days (1052-1207) from visit 2 until the end of study (30th June 2021). Further details are displayed below in Table 4.3.

Table 4-3: Data table for each aetiology subgroup within the ESLD cohort describing the complications of liver disease

	ESLD	ARLD	IMMUNE MEDIATED	NAFLD
N	42	20	14	6
DISEASE SEVERITY				
MELD	12.0 [10.8-15.3]	14.0 [11-17.8]	11.5 [10.8-13.3]	11.5 [8.8-21.3]
UKELD	52.5 [51.0-55.0]	55.0 [52.3-55.8]	52.0 [51.0-53.0]	52.0 [50.3-56.3]
Childs Pugh score	8.0 [6.0-9.0]	9.0 [8.3-9.0]	6.0 [5.8-7.3]	7.5 [5.8-8.3]
COMPLICATIONS OF CLD				
Hepatic encephalopathy	24 (57.1)	20 (100.0)	4 (28.6)	4 (66.7)
Portal Hypertension	38 (90.1)	20 (100.0)	10 (71.4)	6 (100.0)
Ascites	31 (73.8)	20 (100.0)	5 (35.7)	4 (66.7)
- *Diuretics*	24 (57.1)	14 (70.0)	5 (35.7)	3 (50.0)
- *LVP*	9 (21.4)	7 (35.0)	0 (0.0)	1 (16.7)
- *SBP*	10 (23.8)	8 (40.0)	1 (7.1)	1 (16.7)
- *Antibiotic prophylaxis*	10 (100.0)	8 (100.0)	0 (0.0)	1 (16.7)
Varices	25 (59.5)	12 (60.0)	7 (50.0)	4 (66.7)
- *TIPSS*	1 (2.4)	0 (0.0)	1 (7.1)	0 (0.0)
- *1^{o} prophylaxis*	18 (42.9)	12 (60.0)	3 (21.4)	2 (33.3)
Portal vein thrombosis	5 (11.9)	5 (25.0)	0 (0.0)	0 (0.0)
COMORBIDITIES				
Cardiovascular disease	4 (9.5)	2 (1.0)	0 (0.0)	2 (33.3)
Hypertension	13 (31.0)	5 (25.0)	3 (21.4)	5 (83.3)
COPD	5 (11.9)	2 (1.0)	3 (21.4)	1 (16.7)
Diabetes mellitus	12 (28.6)	7 (35.0)	2 (14.2)	3 (50.0)
Insulin dependent	9 (21.4)	5 (25.0)	1 (7.1)	3 (50.0)
Hypercholesterolaemia	5 (11.9)	5 (25.0)	3 (21.3)	2 (33.3)
Chronic kidney disease	8 (19.0)	5 (25.0)	0 (0.0)	3 (50.0)
OUTCOME				
LT during the study	14 (33.3)	6 (30.0)	6 (42.9)	2 (33.3)
LT total (until end of study)	21 (50.0)	12 (60.0)	12 (85.7)	2 (33.3)
On LT waiting list	6 (14.3)	1 (0.5)	1(7.1)	2 (33.3)
Deceased	5 (11.9)	3 (15.0)	1 (7.1)	1 (16.7)

*data presented is as absolute value with (percentage)
** two participants excluded due to other aetiology of ESLD

4.3.2 Measures of muscle mass (ESLD vs. Controls)

Muscle mass, irrespective of the assessment modality, was lower in those with ESLD compared to their healthy controls (Table 4.4). There were significant differences between ESLD and controls, observed in quadricep volume index (348.9±71.6 vs 409.3±107.0 cm3/m2, $p<0.05$), peak quadricep ACSA (53.8±12.0 cm^2 vs 64.8±17.8 cm^2, $p<0.01$) , muscle thickness

(2.1± 0.4 cm vs 2.4±0.5 com p<0.05), and fat free mass (61.9±12.4 kg vs 2.4±0.5 kg, p<0.01).

Interestingly, MAMC did not significantly differ between both groups. (Figure 4.1)

Table 4-4: Mean and S.D. for each mass measurement within the different modalities used to measure muscle mass of the End Stage Liver Disease (ESLD) and healthy control cohorts.

MODALITY	MEASUREMENT	ESLD	CONTROLS		
		Mean [SD]	**Mean [SD]**	**P value**	**% difference between means**
MR QUADRCIEPS N = 39	**Cone volume (cm^3)**	1060 [262.5]	1227 [393.5]	0.06	13.6
	Volume index (cm^3/m^2)	348.9 [71.6]	409.3 [107.0]	<0.05	14.7
	ACSA (cm^2)	53.8 [12.0]	64.8 [17.8]	<0.01	16.9
	L3 SMI* (cm^3/m^2)	41.2 [9.3]	43.7 [8.7]	0.41	5.7
ULTRASOUND N = 42	**Muscle thickness (cm)**	2.1 [0.4]	2.4 [0.5]	< 0.05	12.5
ANTHROPOMETRY N=42	**MAMC (cm)**	27.6 [5.7]	26.5 [4.4]	0.53	-4.2
BIOIMEPEDENCE ANALYSIS N=42	**Fat free mass (kg)**	61.9 [12.4]	51.0 [11.5]	<0.01	-21.4

*N = 32

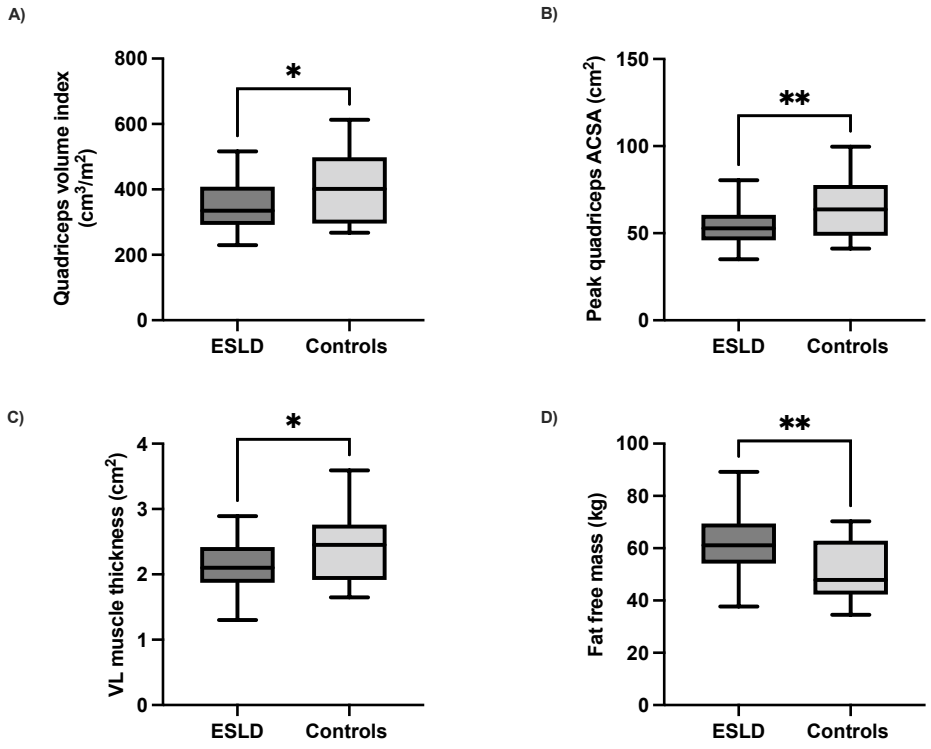


Figure 4-1: Unpaired t test of measures of muscle mass in ESLD compared to a healthy control cohort.

A) Comparison of quadriceps muscle volume index via MRI B) comparison of peak quadriceps ACSA via MRI C) comparison of VL muscle thickness via US and D) comparison of fat free mass via BIA). Data are expressed as median (central horizontal line), 25th and 75th percentiles (box) and the minimum and maximum values (vertical lines). p values as denoted for each figure; * $p<0.05$, ** $p<0.01$.

4.3.3 Measures of muscle quality (ESLD vs. Controls)

The quadriceps IMAT percentage was higher in ESLD compared to healthy controls (10.6±3.5% vs 5.2±1.8%, P<0.0001) (Figure 4.2). Vastus lateralis architecture differed in pennation angle (12.4±3.0 deg vs 16.3±3.9 deg, P<0.0001), however, fascicle length between ESLD and controls did not. Vastus lateralis PCSA was significantly greater in ESLD compared to controls (42.3cm^2 (13.2) vs 58.1cm^2 (19.1), P<0.0001, (Figure 4.3). Furthermore, there was no significant difference in specific force was seen between the two groups (Table 4.5).

Table 4-5: Mean and S.D. for measures of muscle quality in ESLD as compared to healthy controls

	ESLD	CONTROLS	
N	39	18	
MEASURE	**Mean [SD]**	**Mean [SD]**	**P value**
Intermuscular adipose tissue (IMAT) (%)**	10.6 [3.5]	5.2 [1.8]	<0.0001
VL PCSA (cm2)*	42.3 [13.2]	58.1 [19.1]	<0.0001
Pennation angle (degrees)*	12.4 [3.0]	16.3 [3.9]	<0.0001
Fascicle length (cm)*	8.9 [1.4]	8.1 [1.4]	<0.05
Specific force (Nm/cm2) – non-dominant leg**	2.0 [0.5]	2.2 [0.5]	0.17

*n=42, **n=39

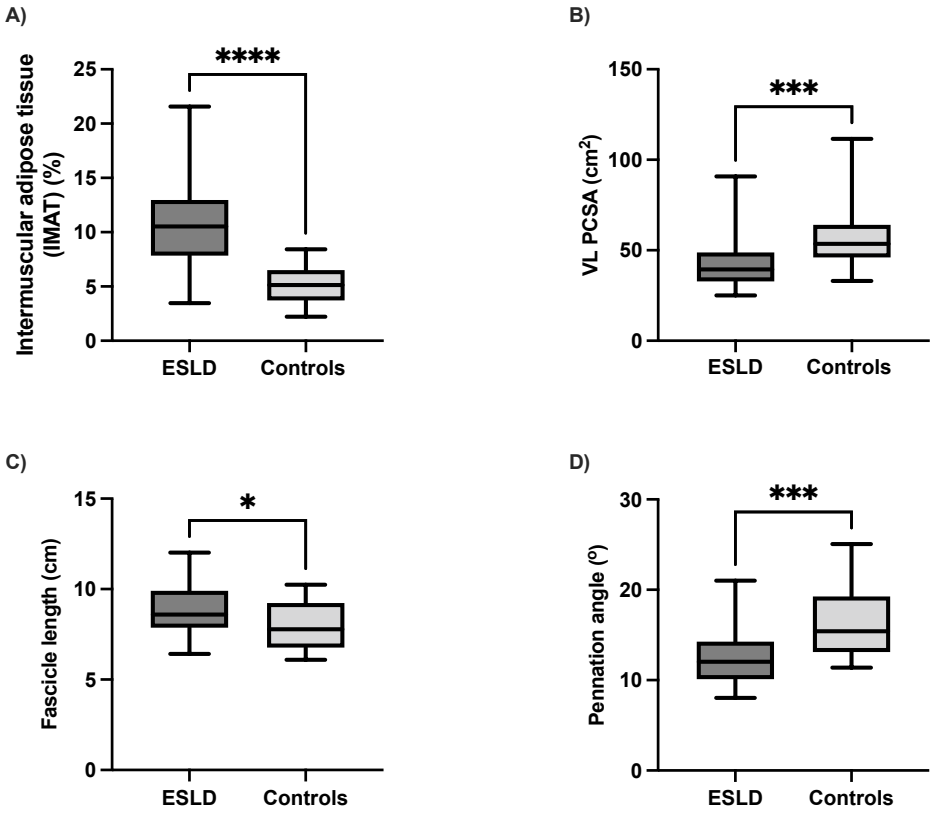


Figure 4-2: Unpaired t test of measures of muscle quality and architecture in ESLD compared to a healthy control cohort.

A) Intermuscular adipose tissue (IMAT) via MRI B) Vastus lateralis physiological CSA (PCSA) C) Fascicle length via ultrasound and D) Pennation angle via ultrasound). Data are expressed as median (central horizontal line), 25^{th} and 75^{th} percentiles (box) and the minimum and maximum values (vertical lines). p values as denoted for each figure; * p<0.05, ** p<0.01, *** p<0.001.

4.3.4 Measures of muscle strength and function

All HGS readings (average, peak, dominant limb, non-dominant limb) were reduced in ESLD compared to control (Figure 4.3) (with the largest difference seen in the non-dominant hands (29.4 vs. 36.6 kg, 19.7% mean difference, p<0.01) (Table 4.6).

Table 4-6: Mean and S.D. for each mass measurement within the different modalities used to measure muscle strength of the End Stage Liver Disease (ESLD) and healthy controls.

	ESLD	CONTROLS		
N	42	18		
	Mean [SD]	**Mean [SD]**	**P value**	**% difference between means**
ANTHROPOMETRY				
HGS average dominant hand	31.6 [8.3]	36.9[10.5]	<0.05	14.4
HGS average non-dominant hand	29.4 [8.0]	36.6 [8.5]	<0.01	19.7
HGS Peak dominant hand	33.0 [8.5]	39.4 [10.7]	<0.05	16.2
HGS Peak non-dominant hand	30.7 [8.2]	38.2 [8.49]	<0.01	19.6
ISOKINETIC DYNAMOMETRY (non-dominant limb)				
Extensor peak torque	100.9 [36.6]	142.5 [51.0]	<0.001	29.2
Extensor peak torque to body weight	115.7 [42.5]	199.2 [60.4]	<0.0001	41.9
Extensor average power	57.6 [23.4]	84.7 [31.6]	<0.01	32.0
Flexor peak torque	*50.9 [24.3]*	*68.2 [23.2]*	*<0.05*	*25.4*
Flexor peak torque to body weight	*57.4 [25.5]*	*95.3 [29.2]*	*<0.0001*	*39.8*
Flexor average power	*26.7 [16.1]*	*43.5 [17.3]*	*<0.001*	*38.6*

Knee extensor strength measures of the quadriceps muscle group were significantly lower between ESLD and controls; absolute peak torque (100.9±36.6 vs 142.5±51.0, p<0.001), peak torque relative to body weight (not adjusted for fluid status) (115.7±42.5 vs 199.2±60.4, p<0.0001), and average power (57.6±23.4 vs 84.7±31.6, p<0.01) (Table 4.6). Knee flexor strength of the hamstrings muscle group demonstrated similarly significant findings of peak torque, peak torque to body weight and average power as described in Table 4.6.

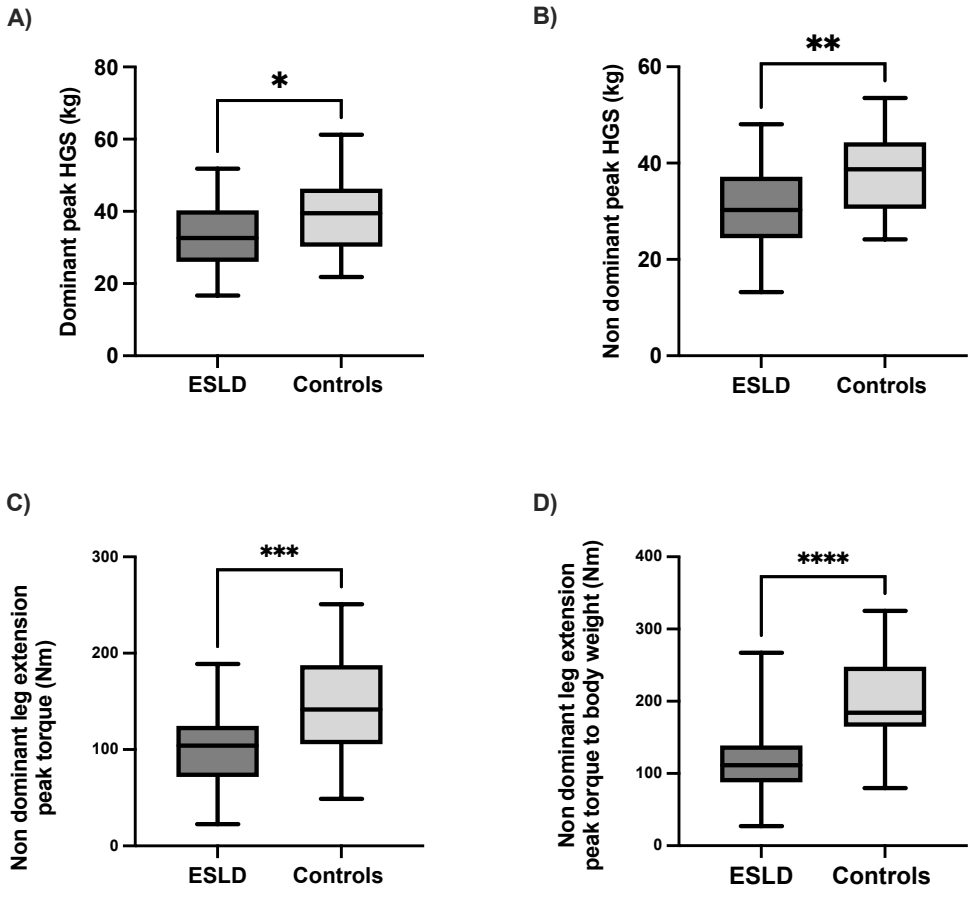


Figure 4-3: Unpaired t test of measures of muscle strength in ESLD compared to their healthy control cohort.

A) Comparison of dominant peak HGS B) comparison of non-dominant HGS C) comparison of non-dominant knee extension peak torque and D) comparison of non-dominant knee extension peak torque to body weight. Data are expressed as median (central horizontal line), 25^{th} and 75^{th} percentiles (box) and the minimum and maximum values (vertical lines). p values as denoted for each figure; * $p<0.05$, ** $p<0.01$, *** $p<0.001$, ****$p<0.0001$.

4.3.5 Composite muscle function measures (ESLD vs. Controls)

There was no significant difference the groups when comparing total SPPB test (gait, balance and chair stands) (Table 4.7). On analysis of the individual values for each domain of gait, balance and chair stand domains, only the time to perform 5 chair stands differed (10.9 secs [9.1-12.8] vs 7.6 secs [5.8-7.7], $p <0.001$) between ESLD and controls (Table 4.7).

Table 4-7: Measures of composite muscle function of the End Stage Liver Disease (ESLD) and control cohorts.

	ESLD	CONTROLS	
N	42	18	
SPPB	**Median [IQR]**	**Median [IQR]**	**P value**
Total score (max 12)	11 [9.5-12]	12 [12.0-12.0]	<0.001
Gait score (max 4)	4.0 [3.0-4.0]	4.0 [4.0-4.0]	<0.001
4 metre walk time (secs)	3.6 [0.1-4.5]	3.3 [3.2 -3.5]	0.11
Balance score (max 4)	4.0 [4.0-4.0]	4.0 [4.0-4.0]	0.30
Chair stand score (max 4)	4.0 [3.0-4.0]	4.0 [4.0-4.0]	<0.001
Chair stand times (secs)	10.9 [9.1-12.8]	7.6 [5.8-7.7]	<0.001
FRAILTY INDICES	**Mean [SD]**	**Mean [SD]**	**P value**
LFI	3.6 [0.5]	2.8 [0.6]	<0.0001

The Liver Frailty Index (LFI) was significantly reduced in ESLD compared with controls with a mean LFI of 3.6±0.5 (*indicating pre-frail status*) as compared to the control mean of 2.8±0.6, p<0.0001, which is classified as robust (Figure 4.4).

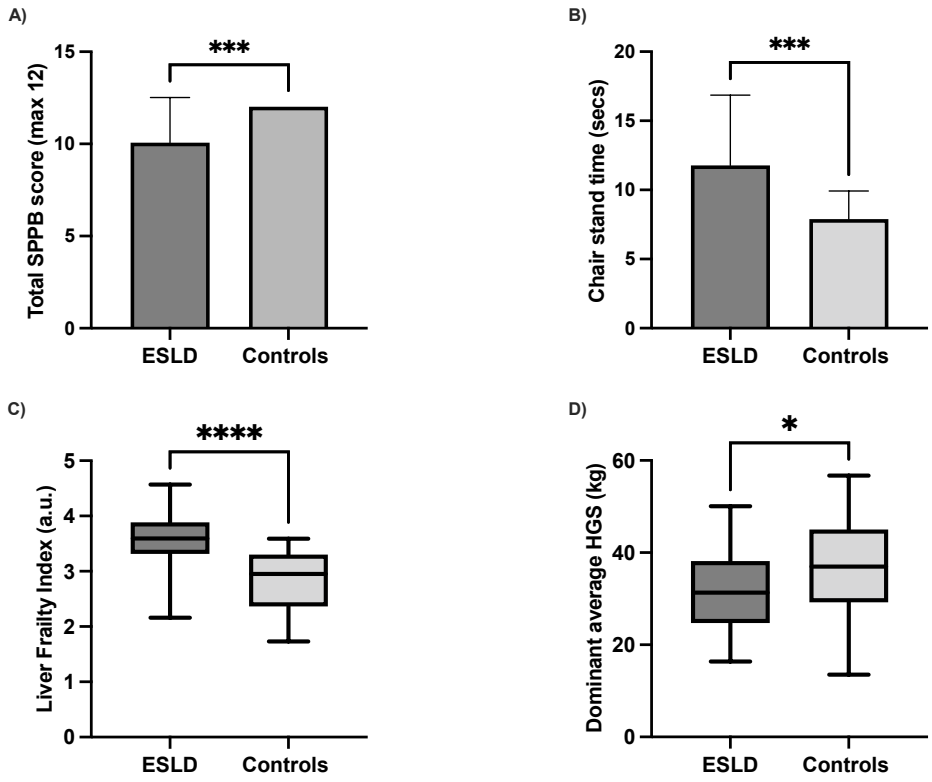


Figure 4-4: Composite functional measures

Composite functional measures: A) Mann Whitney U test of total Short Physical Performance Battery score in ESLD compared to controls. B) Mann Whitney U test chair stand time. Data are expressed as median bars with maximal value (vertical lines). C) Unpaired t test of Liver Frailty Index in ESLD

compared to cohorts and D) dominant average handgrip strength (HGS) in ESLD compared to controls. Data are expressed as median (central horizontal line), 25th and 75th percentiles (box) and the minimum and maximum values (vertical lines). p value denoted for each figure; * p<0.05, ** p<0.01, *** p<0.001.

4.3.6 Physical activity

The overall daily activity was lower in ESLD than their controls (18.4±3.7 vs 29.2±8.9 mg, p<0.001). The intensity gradient was reduced in ESLD as compared to the control (-2.8±0.2 vs -2.4±0.3). The ESLD cohort also spent less time at a moderate – vigorous physical activity level (48.3±18.2 mins/day vs 104.8±40.9 mins/day) and had lower number of mean days with a 30 minute or 15 mins walk at a moderate activity level (>100mg) (Figure 4.5). Finally, measures of inactive days and inactive time did not differ between these groups (Table 4.8).

Table 4-8: Mean and S.D. for the measures of accelerometery in ESLD and controls

	ESLD	CONTROLS	
N	42	18	
	Mean [SD]	**Mean [SD]**	**P value**
ACCELEROMETRY			
Overall daily activity (mg)	18.4 [3.7]	29.2 [8.9]	<0.0001
Intensity gradient	-2.8 [0.2]	-2.4 [0.3]	<0.0001
Total MVPA (>100mg) (mins/per day)	48.3 [18.2]	104.8 [40.9]	<0.0001
Days with 30 min walk (>100mg, n)	0.4 [1.4]	1.6 [1.5]	<0.05
Days with 15 min walk (>100mg, n)	0.9 [1.8]	3.8 [1.7]	<0.0001
Total inactive days	8.8 [3.6]	7.3 [4.1]	0.18
Total inactive time (mins)	785.6 [134.8]	710.4 [124.9]	<0.05
Sleep window length (hours)	7.6 [1.8]	8.2 [0.6]	0.25
Actual sleep time (hours)	5.9 [1.4]	7.1 [0.8]	<0.01
Sleep efficiency (%)	77.7 [7.3]	87.1 [6.8]	<0.0001

Furthermore, the ESLD cohort had a lower sleep time and reduced sleep efficiency compared to healthy controls (5.9±1.4 hours vs 7.1±0.8 hours, p<0.01) and 77.7±7.3 % vs 87.1±6.8 %, p<0.001 respectively) (Figure 4.6).

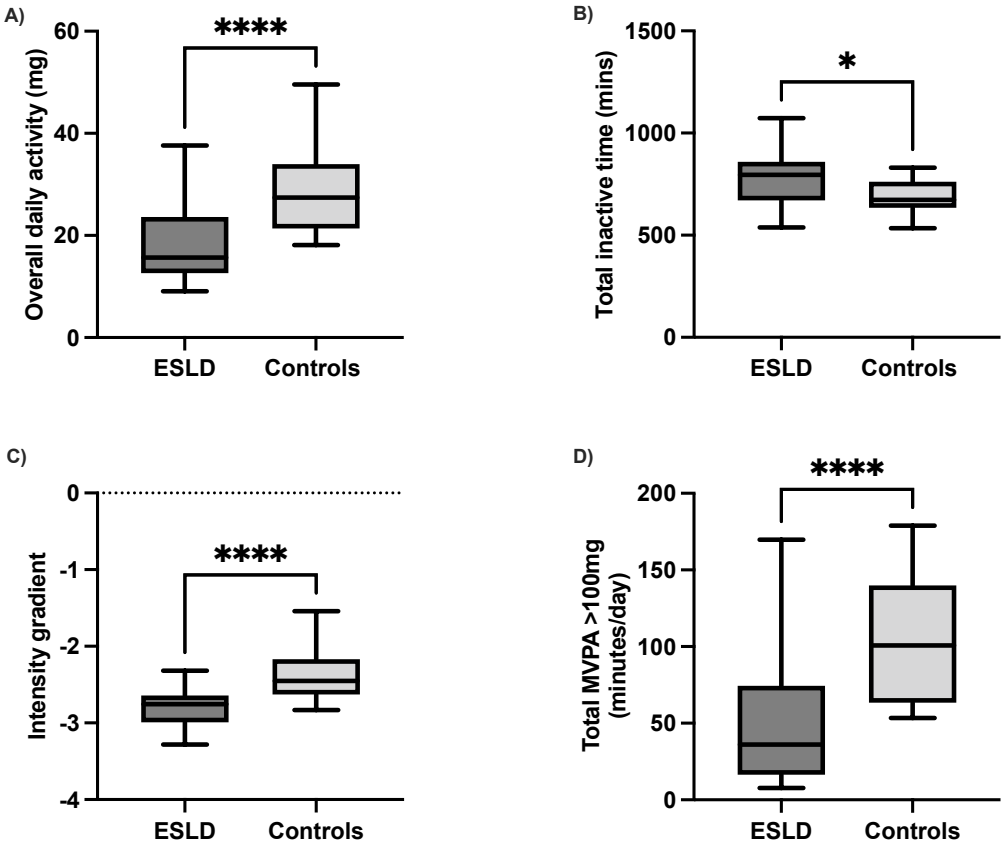


Figure 4-5: Unpaired t test of physical activity variables in ESLD compared to controls.

A) Overall daily activity B) Total inactive time C) Intensity gradient and D) Total moderate – vigorous physical activity >100mg. Data are expressed as median (central horizontal line), 25th and 75th percentiles (box) and the minimum and maximum values (vertical lines). p value denoted for each figure; * p<0.05, ** p<0.01, *** p<0.001.

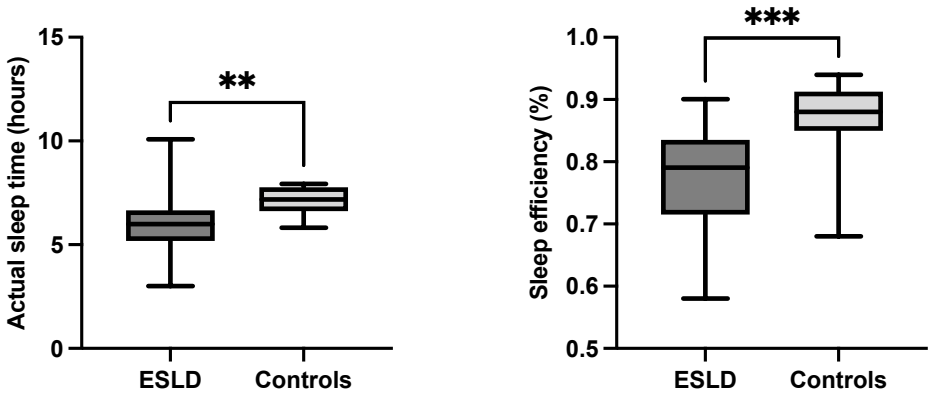


Figure 4-6: Unpaired t test of measures of sleep variables in ESLD compared to controls

Data are expressed as median (central horizontal line), 25th and 75th percentiles (box) and the minimum and maximum values (vertical lines). p value denoted for each figure; * p<0.05, ** p<0.01, *** p<0.001, ****P<0.0001.

4.3.7 Effect of age, sex, aetiology and disease severity (ESLD sub-groups vs. Controls)

The data were next reclassed in subgroups of age, disease severity based on UKELD, aetiology and sex (Table 4.1).

4.3.7.1 Disease aetiology

There were no differences in the measures of muscle mass between disease aetiologies or from each aetiology compared to controls. The mean IMAT percentage in all aetiologies of ESLD were higher than controls (controls 5.2±1.8% vs ArLD 11.3±3.4%, p <0.0001, vs immune mediated 10.34±3.6%, p<0.001, vs NAFLD 10.4±3.4%, p<0.01 respectively). Knee extensor peak torque was significant lower in the ArLD subgroup than controls (96.7±30.3 Nm vs 142.5±51.1 Nm, p<0.001) however peak dominant HGS was significantly lower in NAFLD subgroup to controls. (26.2±8.6 kg vs 39.4±10.7 kg, p<0.05) (Figure 4.7).

Controls demonstrated lower LFI score than ArLD subgroups, immune mediated and NAFLD subgroups (ArLD 3.7±0.6 vs 2.8±0.6, p<0.0001 vs immune mediated 3.4±0.5 vs 2.8±0.6, p<0.05, vs NAFLD 3.8±0.2 vs 2.8±0.6, p<0.01). Only those with ArLD demonstrated a significantly lower overall daily activity level (14.2±4.6 mg vs 29.2±8.9 mg, p<0.0001) and greater total inactive time between the ArLD (815.1±140.2mins vs 685.6±82.8 mins, p<0.05) compared to controls. There was significant difference between overall daily activity in the ArLD and immune mediated subgroups (14.2±4.6 mg vs 24.7±6.4 mg, p<0.01).

Furthermore, sleep efficiency was lower in all aetiologies of ESLD to controls (ArLD 78.5±8.4% vs 87.1±6.7%, p<0.01, immune mediated 78.8±5.8% vs 87.1±6.7%, p<0.05, and NAFLD 72.3±6.1% vs 87.1±6.7%, p<0.05 respectively).

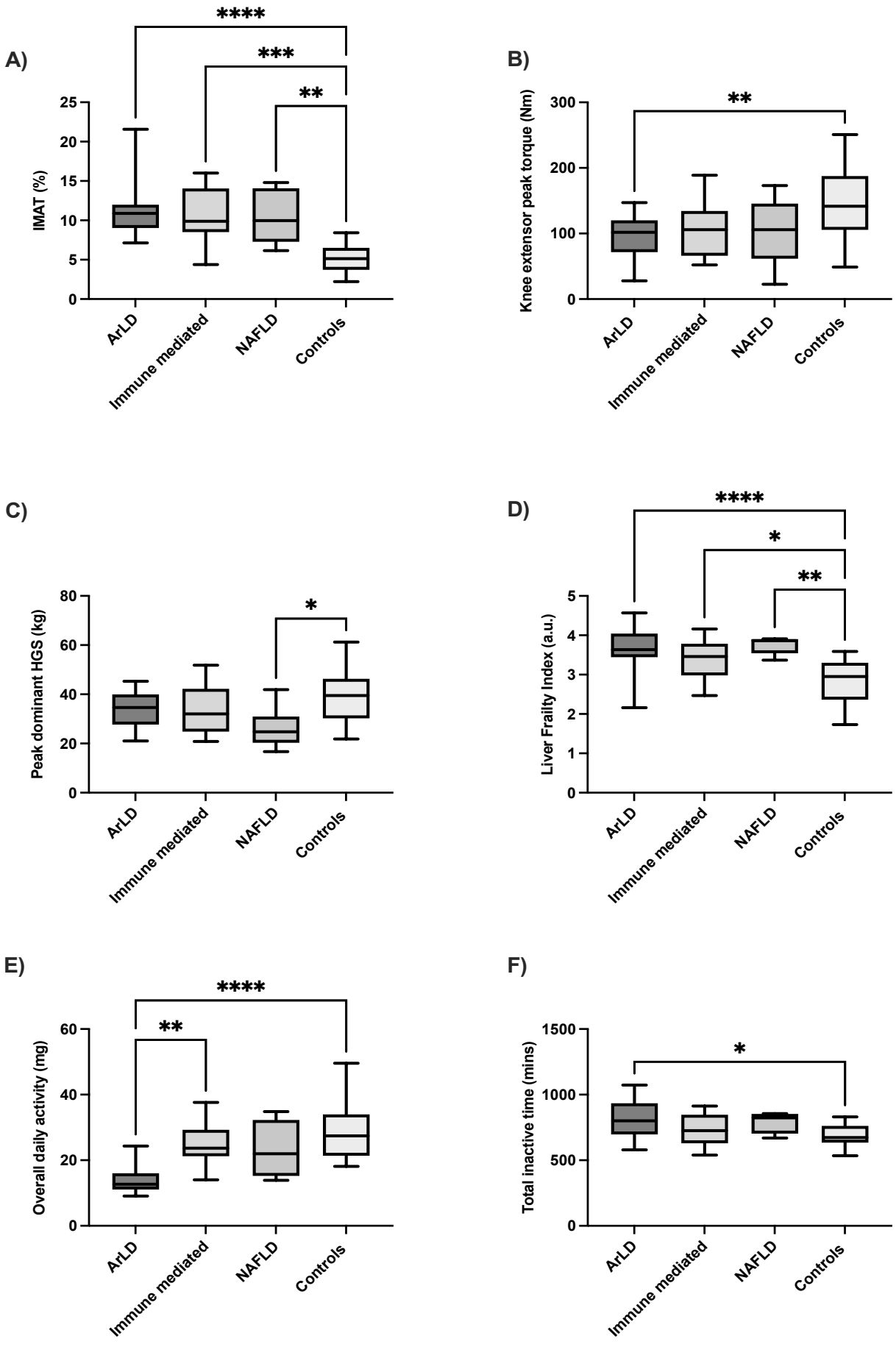

A)
IMAT (%)
****
***
**
B)
Knee extensor peak torque (Nm)
**
C)
Peak dominant HGS (kg)
*
D)
Liver Frailty Index (a.u.)
****
*
**
E)
Overall daily activity (mg)
****
**
F)
Total inactive time (mins)
*
ArLD
Immune mediated
NAFLD
Controls

Figure 4-7: One-way ANOVA of ESLD subgroups of disease aetiology compared to controls;

A) Quadriceps gender subgroups, B) knee extensor peak torque, C) dominant peak handgrip strength, D) Lover frailty index, E) overall daily activity and F) total inactive time for the ESLD aetiologies compared to controls. Data are expressed as median (central horizontal line), 25^{th} and 75^{th} percentiles (box) and the minimum and maximum values (vertical lines). p value denoted for each figure; * p<0.05, ** p<0.01, *** p<0.001, and ****p<0001.

4.3.7.2 Disease severity

Figure 4.9 shows that those with a UKELD of 50-56 demonstrated a lower peak quadriceps ACSA (51.9±11.8cm^2 vs 64.8±17.8 cm^2, p<0.05) and lower extensor peak torque (102.9±36.4 Nm vs 142.5±51.1 Nm, p< 0.01). There were no differences between the other measures of muscle mass or strength (HGS) between disease severity groups or compared to controls. This may of course be explained by the fact the majority of the patients were in the UKELD 50-56 category, with only 5 in the most severe sub-group UKDL >56. However, despite the sample size all disease severity subgroups showed a higher percentage IMAT compared to controls (UKELD ≤49 10.2±4.0% vs controls 5.2±1.8%, p<0.01; UKELD 50-55 10.5±3.7% vs controls 5.2±1.8% p<0.0001; UKELD≥56 11.1±2.9% vs controls 5.2±1.8%, p<0.001). Overall daily activity was also reduced in subgroups of disease severity (UKELD≤49, UKELD 50-55, and UKELD≥56) to controls (19.0±6.8mg vs 29.2±8.9mg. p<0.05, 19.0±7.6mg vs ±29.2±8.9mg., p<0.001, and 12.3±4.8mg vs 29.2±8.9mg., p<0.01) (Figure 4.8).

All subgroups of ESLD disease severity demonstrated greater LFI scores as compared to controls, implying greater frailty (UKELD ≤49 3.6±0.7 vs 2.8±0.6 p<0.01, UKELD 50-55 3.5±0.5 vs 2.8±0.6, p<0.0001 and UKELD ≥56 3.7±0.3 vs 2.8±0.6, p<0.01 respectively). Chair stand times were similarly significantly greater in all ESLD disease severity subgroups compared to controls. (UKELD≤49 13.0±5.1 vs 7.9±2.0 secs, p<0.05, UKELD 50-55 11.2±4.8 secs vs 7.9±2.0

secs, p<0.05, and UKELD≥56 13.2±4.2 secs vs 7.9±2.0 secs, p<0.05). Finally, there was no difference between the sleep efficiency or daily actual sleep between the disease severity subgroups or when compared to controls.

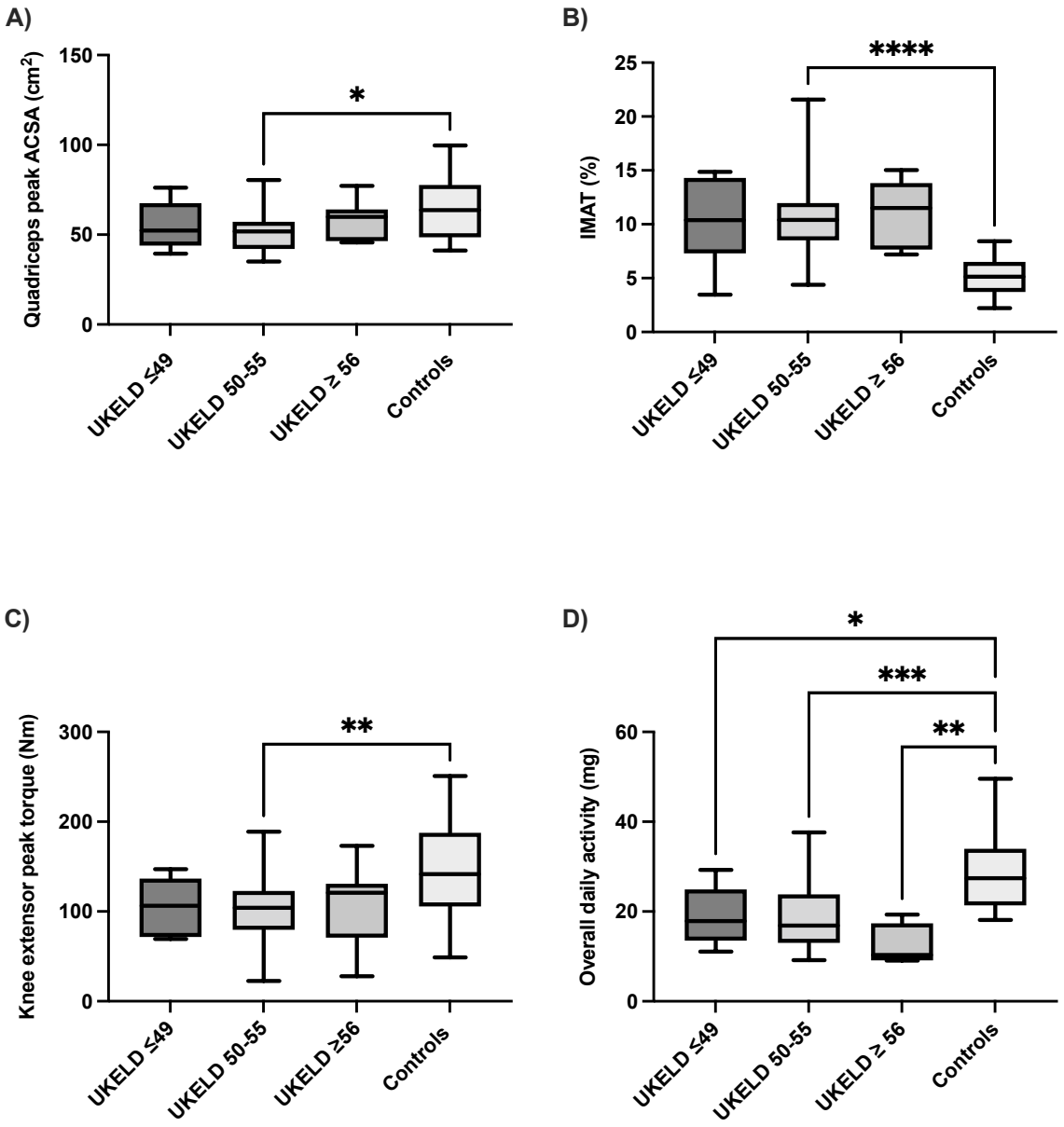


Figure 4-8: One-way ANOVA of ESLD subgroups of disease severity by UKELD to controls

A) Quadriceps peak ACSA, B) IMAT, C) knee extensor peak torque and D) overall daily activity. Data are expressed as median (central horizontal line), 25th and 75th percentiles (box) and the minimum and maximum values (vertical lines). p value denoted for each figure; * p<0.05, ** p<0.01, *** p<0.001, and ****p<0001.

There was a significant difference in the mean serum ammonia concentration of those with ESLD and HE compared to those with ESLD and no HE (85.6± 47.0 vs 57.2±21.5μmol/L) (Figure 4.9). Peak quadriceps ACSA in those with and without HE and ESLD was reduced compared to controls (ESLD with HE 53.7±11.4cm^2 vs 64.8±17.8 cm^2, $p<0.05$; ESLD without HE 53.3±12.9cm^2 vs 64.8±17.8 cm^2, $p<0.05$). Knee extensor peak torque was lower in both those with and without HE and ESLD compared to controls (ESLD with HE 101.5±37.7 Nm vs 142.5±51.1Nm, $p<0.01$; ESLD without HE 101.8±36.5 vs 142.5±51.1Nm, $p<0.05$). Non-dominant HGS was also weaker in those with and without HE compared to controls (ESLD with HE 29.4±8.0 kg vs 38.2±8.49 kg, $p<0.05$; ESLD without HE 30.7±8.3 kg vs 38.2±8.49 kg, $p<0.05$). Habitual daily activity was reduced in those with and without HE compared to controls (16.7±5.4 mg vs 29.2±8.9mg, $p<0.0001$ and 21.2±8.9mg vs 29.2±8.9mg, $p<0.01$ respectively). The control cohort had a lower LFI score compared to those with and without HE (ESLD with HE 3.6±0.5vs 2.8±0.6, $p>0.001$; ESLD without HE 3.5±0.5 vs 2.8±0.6, $p<0.001$) (Figure 4.9).

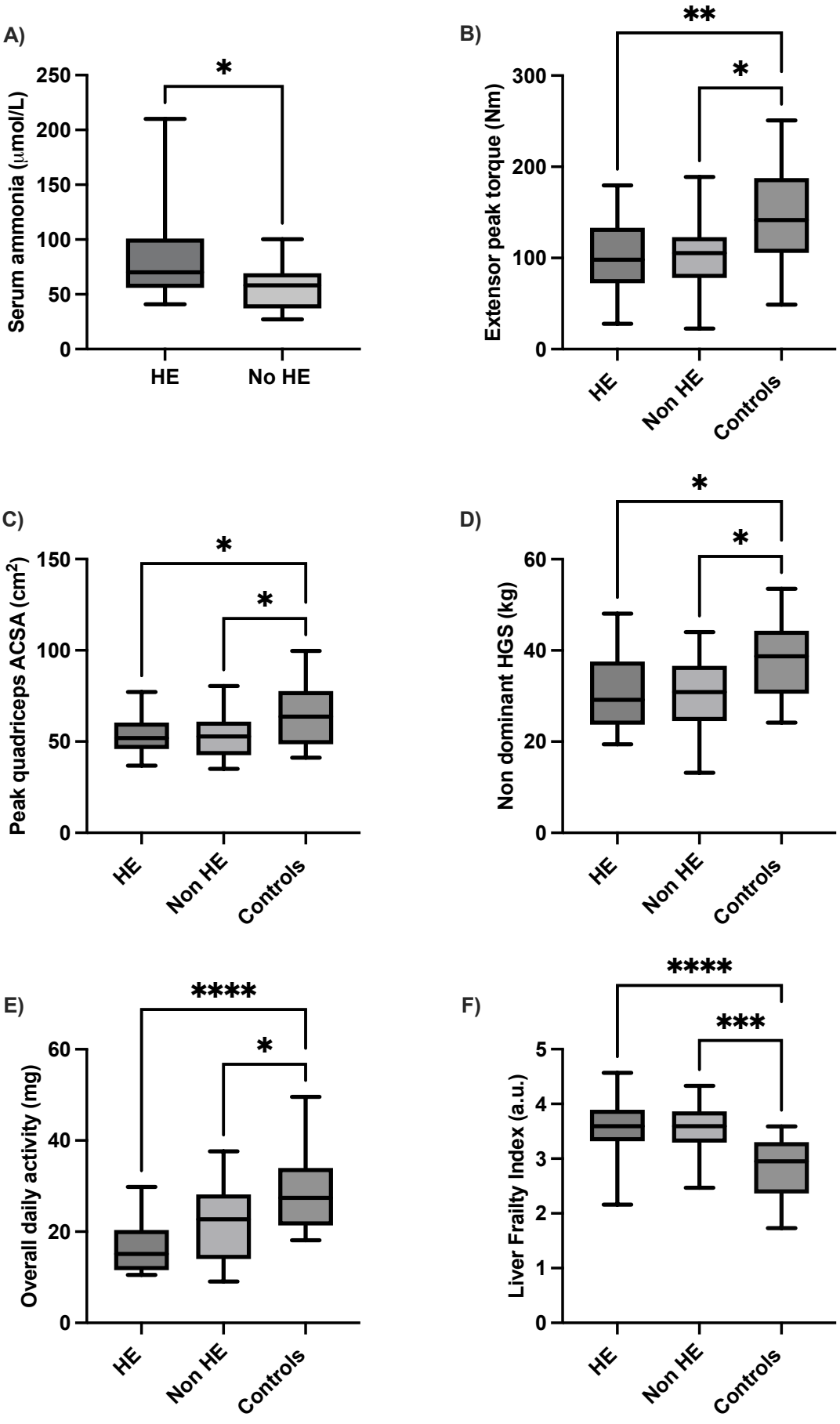

A)
Serum ammonia (µmol/L)
250
200
150
100
50
0
*
HE
No HE
B)
Extensor peak torque (Nm)
300
200
100
0
**
*
HE
Non HE
Controls
C)
Peak quadriceps ACSA (cm²)
150
100
50
0
*
*
HE
Non HE
Controls
D)
Non dominant HGS (kg)
60
40
20
0
*
*
HE
Non HE
Controls
E)
Overall daily activity (mg)
60
40
20
0
****
*
HE
Non HE
Controls
F)
Liver Frailty Index (a.u.)
5
4
3
2
1
0
****
***
HE
Non HE
Controls

Figure 4-9: Comparison of HE subgroups

A) Unpaired t test of serum ammonia in ESLD with HE compared to ESLD without HE subgroup to controls; One-way ANOVA of ESLD HE and no HE subgroups compared to controls B) extensor peak torque, C) quadriceps ACSA, D) non-dominant HGS, E) overall daily activity and F) liver frailty index. Data are expressed as median (central horizontal line), 25^{th} and 75^{th} percentiles (box) and the minimum and maximum values (vertical lines). p value denoted for each figure; * p<0.05, ** p<0.01, *** p<0.001, and ****p<0001.

A further subgroup analysis via presence and severity of ascites are shown in Figure 4.10. Peak quadriceps ACSA was reduced in those with diuretic refractory ascites compared to controls (45.6±6.8 cm2 vs 64.8±17.8 cm2, p< 0.05). Those with diuretic refractory ascites had a lower gait score (2.6±1.5 vs 4.0±0.0, P<0.01) and greater chair stand time (12.9±8.0 secs vs 7.9±2.0 secs, p<0.05) when compared to controls. Both diuretic refractory ascites and diuretic controlled ascites has a reduced quadriceps extensor peak torque against controls (92.0±22.0 Nm ± 142.5±51.0 Nm, p<0.05 and 102.7±34.3 Nm vs ±142.4±51.0 Nm, p<0.05).

Habitual daily activity was reduced in those with diuretic refractory ascites compared to controls (13.5±1.7 mg vs 29.2±8.9 mg, p<0.001) and compared to those with ESLD without ascites (18.0±7.4 mg vs 24.7±6.8 mg, p<0.05). Those with diuretic controlled ascites also has a lower average daily activity (18.0±7.4 mg vs 29.2±8.9 mg, p<0.01) (Figure 4.10).

The intensity gradient of the daily physical activity was lower in those with diuretic refractory ascites compared to controls (-3.2±0.2 vs -2.4±0.3, p<0.0001) and compared to those with ESLD without ascites (-3.2±0.2 vs -2.6±0.1, p<0.01). Those with diuretic controlled ascites also has a lower average gaily activity (-2.8±0.2 vs -2.4±0.3, p<0.0001) (Figure 4.10).

The time spent at a moderate to vigorous activity level was also lower in those with diuretic refractory ascites compared to controls (17.4±7.2 minutes/day vs 104.8±40.9 minutes/day, $p<0.0001$) and compared to those with ESLD without ascites (17.4±7.2 minutes/day vs 85.7±41.9 minutes/day, $p<0.01$). Those with diuretic controlled ascites also has a lower average daily activity (46.2±33.7 minutes/day vs 104.8±40.9 minutes/day, $p<0.01$) (Figure 4.10).

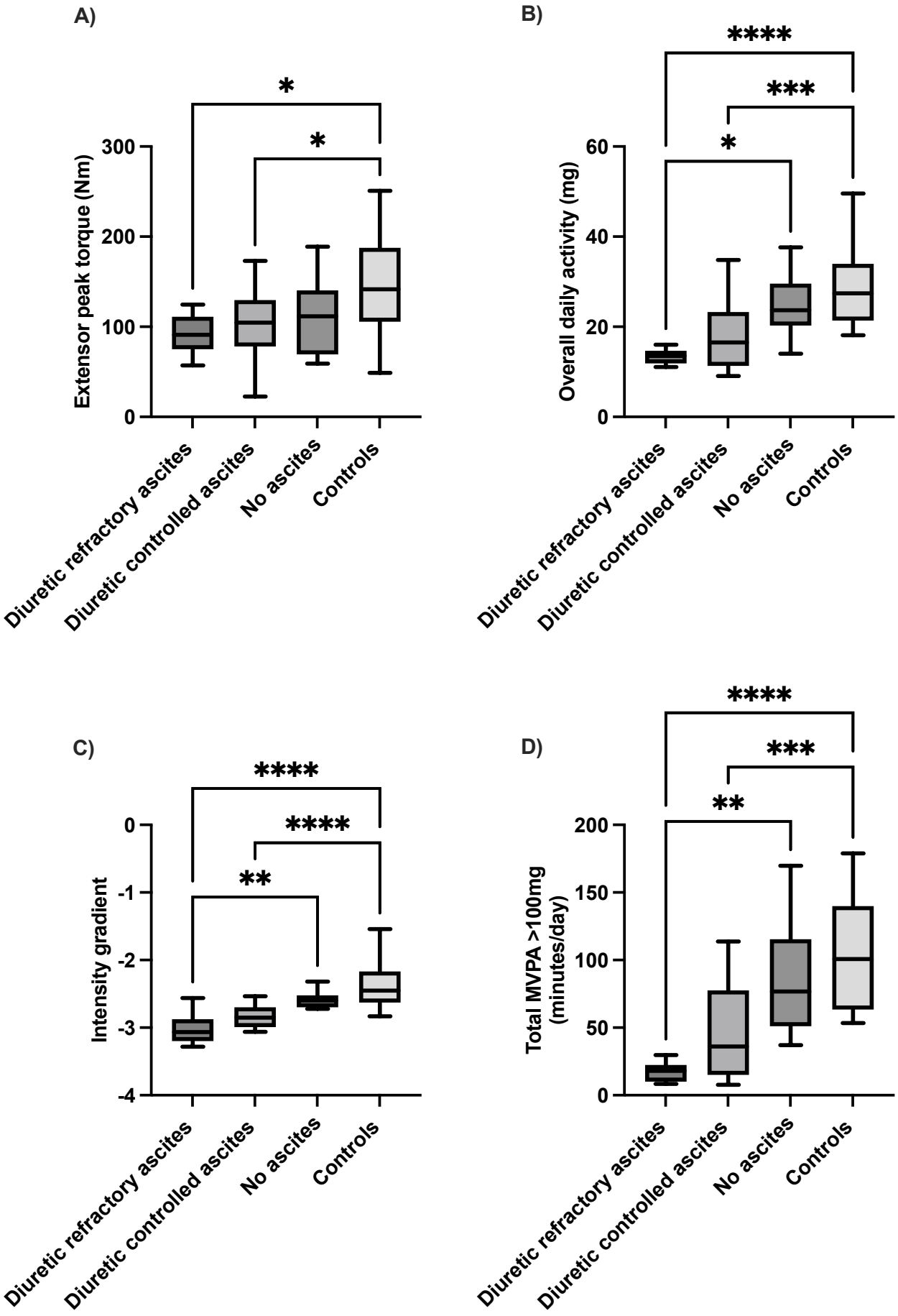

A)
Extensor peak torque (Nm)
300
200
100
0
*
*
Diuretic refractory ascites
Diuretic controlled ascites
No ascites
Controls
B)
Overall daily activity (mg)
60
40
20
0
****
***
*
Diuretic refractory ascites
Diuretic controlled ascites
No ascites
Controls
C)
Intensity gradient
0
-1
-2
-3
-4
****
****
**
Diuretic refractory ascites
Diuretic controlled ascites
No ascites
Controls
D)
Total MVPA >100mg (minutes/day)
200
150
100
50
0
****
***
**
Diuretic refractory ascites
Diuretic controlled ascites
No ascites
Controls

Figure 4-10: One-way ANOVA of ESLD ascites subgroups compared to controls

A) knee extensor peak torque, B) overall daily activity, C) intensity gradient, and D) total MVPA. Data are expressed as median (central horizontal line), 25^{th} and 75^{th} percentiles (box) and the minimum and maximum values (vertical lines). p value denoted for each figure; * p<0.05, ** p<0.01, *** p<0.001, and ****p<0001.

4.3.7.3 Age

Figure 4.12 shows that peak quadriceps ACSA in those with ESLD aged 60-71 years was lower than controls (50.4±10.0 cm^2 vs 64.8±17.8 cm^2, p<0.05) (Figure 4.11). All subgroups of age showed a great difference of IMAT percentage compared to controls (ESLD 20-49 yrs. 9.7±3.3% vs 5.2±1.8%, p<0.01; 50-59 yrs 10.9±2.8% vs 5.2±1.8%, p<0.0001; 60-71 yrs 0.8±4.5% vs 5.2±1.8%, p<0.0001) (Figure 4.12). Knee extensor peak torque was weaker in older patients with ESLD as compared to controls (ESLD aged 50-59 yrs. 105.3±30.9 Nm vs 142.5±51.1Nm, p<0.05 and ESLD aged 60-71 yrs. 85.5±26.5 Nm vs 142.5±51.1Nm, p<0.01), however there was no difference with HGS. Similarly, older age groups 50-59 years and 60-71 years had a reduced overall daily physical activity compared to controls (ESLD aged 50-59 yrs. 15.9±7.0mg vs p<0.001, and ESLD aged 60-71 18.1±6.7mg, p<0.01 respectively). Furthermore, sleep efficiency was lower in ESLD aged 50-59 years and 60-71 years compared to controls (ESLD 50-59yrs, 77.8±7.4 vs 87.1±6.7%, P<0.01 and ESLD 60-71 yrs., 76.8±8.1 vs 87.1±6.7%, p<0.01 respectively).

All age subgroups of ESLD had a greater LFI score compared to controls (controls vs 20-49 years 3.5±0.6 vs 2.8±0.6, p<0.05, 50-59 years 3.5±0.5 vs 2.8±0.6, p<0.01, and 60-71 years 3.7±0.5, p<0.001 respectively). Chair stand times were significantly greater in the lower ESLD age subgroups compared to control vs (20-49 years mean 12.9±6.7 secs vs 7.9±2.0 secs, p<0.05 and 50-59 years 11.9±4.3 secs vs 7.9±2.0 secs, p<0.05).

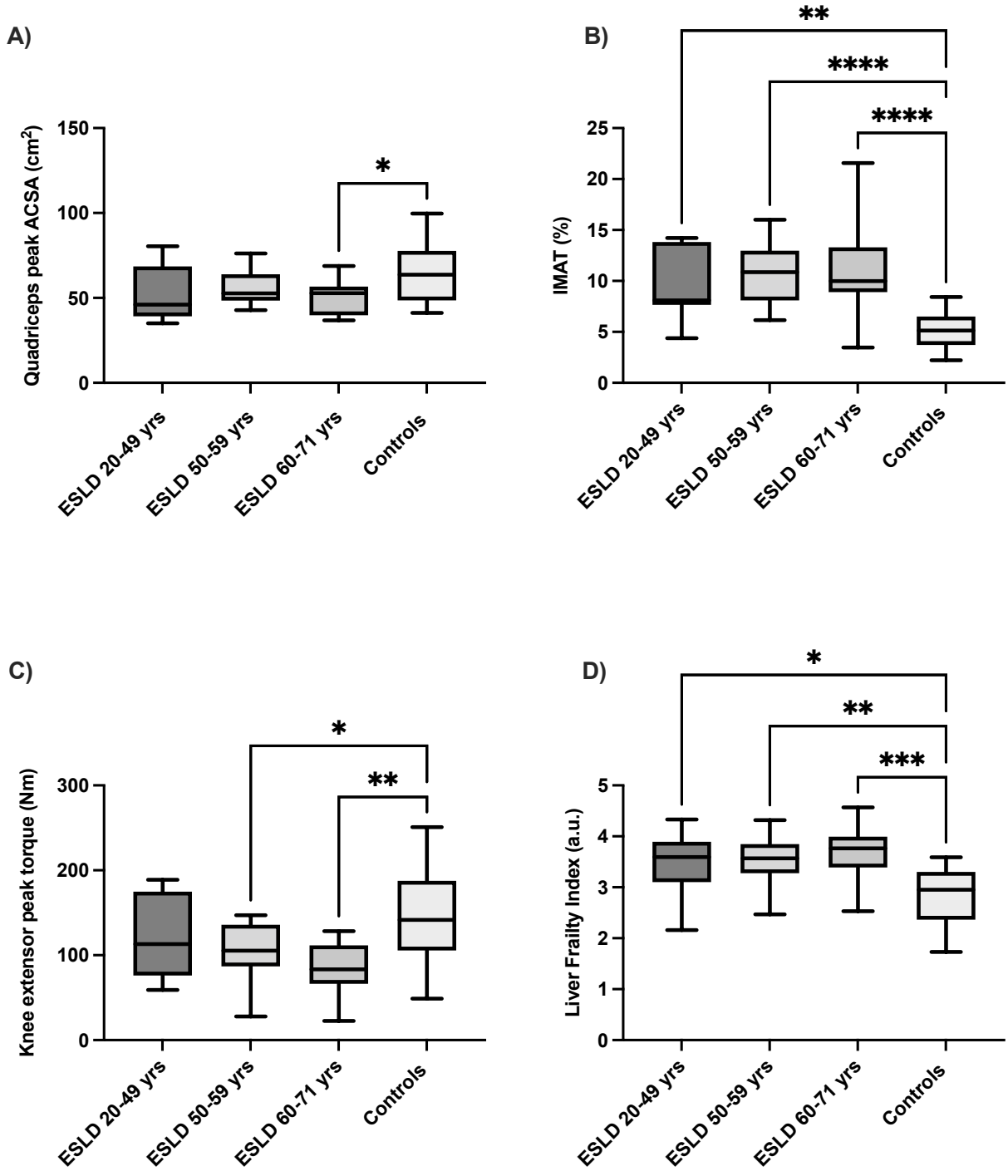


Figure 4-11: One-way ANOVA of ESLD age subgroups compared to controls

A) quadriceps ACSA, B) IMAT, C) Knee extensor peak torque, and D) liver frailty index. Data are expressed as median (central horizontal line), 25th and 75th percentiles (box) and the minimum and maximum values (vertical lines). p value denoted for each figure; * p<0.05, ** p<0.01, *** p<0.001, and ****p<0001

4.3.7.4 Sex

Quadriceps peak ACSA (74.0±15.6 cm^2 vs 58.4±11.5 cm^2, p<0.01), quadricep volume index (462.9±95.2 cm^3/m^2vs 370.0±70.8 cm^3/m^2, p<0.01) and vastus lateralis muscle thickness (2.7±0.5 cm vs 2.1±0.4 cm, p<0.01) were significantly higher in male controls compared to male ESLD (Figure 4.13). Quadriceps peak ACSA was the only measure with a significant difference between sexes (ESLD male 74.0±15.6 cm^2 vs ESLD female 46.5±8.9 cm^2, p<0.05). The mean IMAT percentage was greater in both male and females of ESLD compared to gender matched controls (males 9.7±4.0% vs 4.7±1.5%, p<0.001; females 11.9±2.2% vs 5.9±1.9%, p<0.001 respectively) (Figure 4.12). Knee extensor peak torque was lower in ESLD males compared to control males (115.7±34.3 Nm vs 162.8±54.1 Nm, p <0.01) and peak dominant HGS were higher in ESLD males compared to control males. Both knee extensor peak torque and peak dominant HGS was greater in ESLD males to ESLD females (115.7±34.3) between ESLD males and ESLD females (Figure 4.12).

Furthermore, LFI of male and female genders differed from their respective controls (male ESLD 3.5±0.5 vs male controls 3.0±0.5, p<0.05, female ESLD 3.7±0.5 vs female controls 2.6±0.6, p<0.001) (Figure 4.12).

In contrast to LFI, chair stand time only differed in females (ESLD 13.4±4.6 secs vs controls 6.8±1.1, p<0.01). Both ESLD males and females had a lower overall daily activity compared to their respective controls (ESLD males vs control males) (ESLD females vs control females) There was a reduced sleep efficiency between both genders of ESLD compared to controls (ESLD males vs control males, 77.5±7.0% vs 85.9±8.1%, p<0.05; ESLD female vs controls, 78.4±7.8 vs 89.4±2.5%, p<0.05).

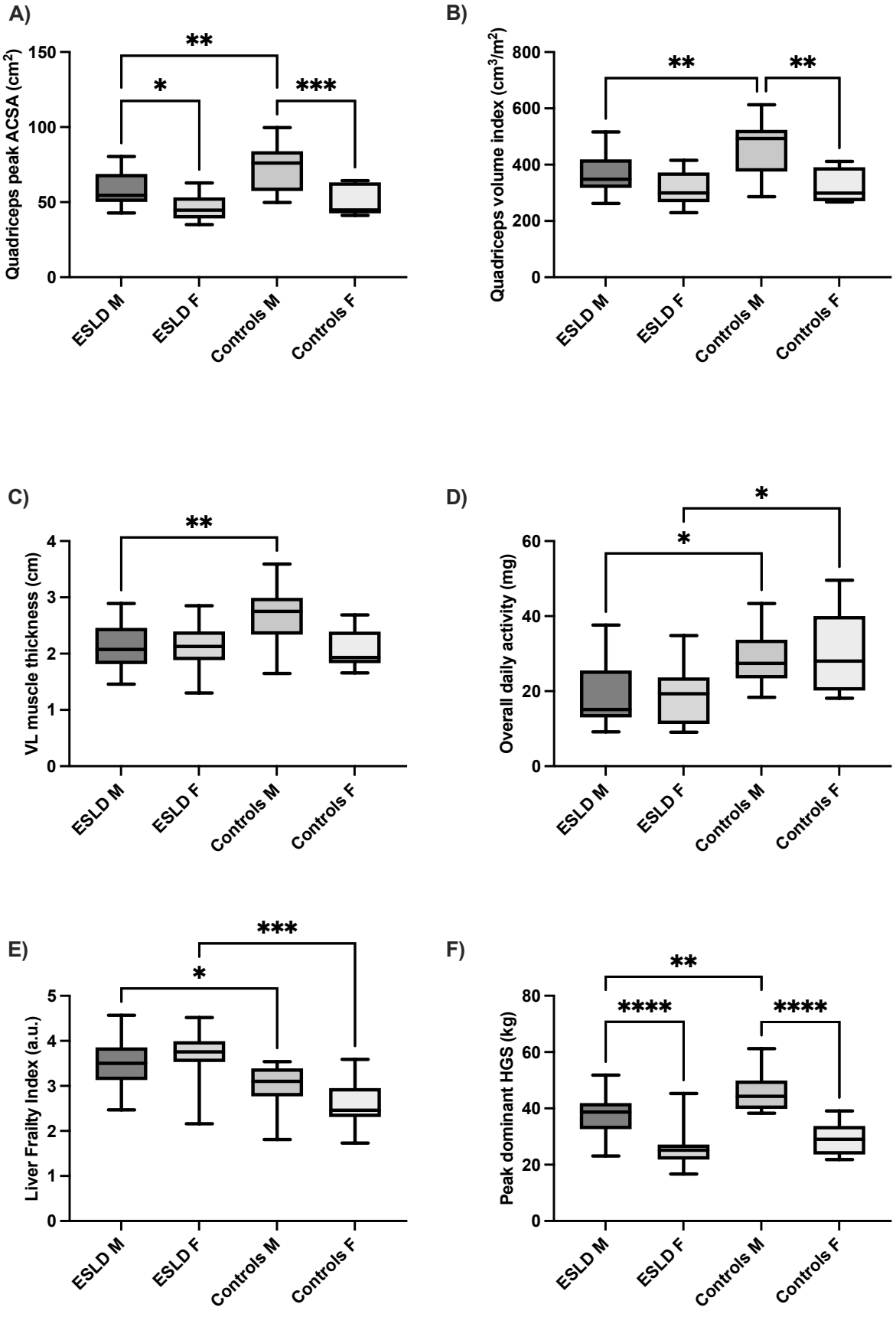

A)
Quadriceps peak ACSA (cm²)
150
100
50
0
**
*
***
ESLD M
ESLD F
Controls M
Controls F
B)
Quadriceps volume index (cm³/m²)
800
600
400
200
0
**
**
ESLD M
ESLD F
Controls M
Controls F
C)
VL muscle thickness (cm)
4
3
2
1
0
**
ESLD M
ESLD F
Controls M
Controls F
D)
Overall daily activity (mg)
60
40
20
0
*
*
ESLD M
ESLD F
Controls M
Controls F
E)
Liver Frailty Index (a.u.)
5
4
3
2
1
0
***
*
ESLD M
ESLD F
Controls M
Controls F
F)
Peak dominant HGS (kg)
80
60
40
20
0
**
****
****
ESLD M
ESLD F
Controls M
Controls F

Figure 4-12: Comparison of sex groups of ESLD and controls

One -way ANOVA of A) quadriceps peak ACSA, B) quadriceps volume index C) vastus lateralis muscle thickness, D) overall daily activity, E) Liver frailty index, and F) peak dominant handgrip strength. Data are expressed as median (central horizontal line), 25^{th} and 75^{th} percentiles (box) and the minimum and maximum values (vertical lines). p value denoted for each figure; * $p<0.05$, ** $p<0.01$, *** $p<0.001$, and ****$p<0001$

4.4 Discussion

This prospective study extensively evaluated multi-modal assessments of muscle mass, strength and function in patients with ESLD, with health age/sex matched controls for comparison. The primary aim of this study was to evaluate differences between variables of muscle mass, strength and performance in those with ESLD compared to a matched healthy control cohort. However, in addition, we wanted to explore the impact of disease aetiology, disease severity, sex and age upon the above measures. The demographic data show that the study has a good representation of the current prevalence of UK liver disease, with a similar average age and leading aetiology to those awaiting LT in the UK (475). This ArLD sub group had more advanced disease than the other groups with a higher rate of complications of ESLD (i.e. HE, portal hypertension and ascites) and higher UKELD. The NAFLD group had the greater prevalence of comorbidities, in particular diabetes. Both of these findings are expected and in keeping with previous literature and clinical findings within those with liver disease (162, 165, 476, 477).

This study demonstrates that patients with ESLD have compromised muscle mass, poorer muscle quality and reduced muscle strength and function in comparison to age and sex matched healthy controls. In contrast to the majority of research in the field of hepatology, the work in this thesis extensively examined muscle mass in the trunk, lower and upper limbs.

Lower limbs mass measures including MRI quadricep volume, volume index, peak quadricep ACSA, and US vastus lateralis muscle thickness were all significantly lower in ESLD compared to controls. However, no difference in MAMC was seen in this study. MAMC remains one of the most utilised measures of muscle mass within clinical practice, however our findings suggest that it may not demonstrate early sensitivity to changes in muscle mass compared to L3 SMI and even more so in quadricep muscle groups. It is well established that as one of the largest muscle groups in the body, the quadriceps are highly susceptible to the earliest changes of muscle loss and sarcopenia (436-439). Research in primary age-related sarcopenia has demonstrated the concept of preserved upper limb mass in contrast to a greater lower limb muscle mass loss (306, 436). Collectively, this suggests that the quadriceps muscle groups should perhaps be of greater focus in ESLD.

The current definition of sarcopenia in those with ESLD is defined by sex specific cut offs for L3 SMI of < 50 cm2/m2 in men and < 39 cm2/m2 in women (219, 325), rather than lower limb measures of muscle mass. Interestingly, due to technical issues in the MRI image acquisition, there was an incomplete dataset for L3 SMI measure of muscle mass. However, L3 SMI was still performed in >75% of the cohort with sex differences in L3 SMI for both ESLD and controls were seen. These results, again, suggest that quadriceps measures of muscle mass may be more sensitive at identifying changes in muscle mass than L3 SMI in this cohort, in keeping with research findings in age related sarcopenia (306, 436). The results further support the need for sex specific cut-offs in measures of muscle mass in ESLD.

Aside from muscle mass, the quality of the muscle is an important consideration in sarcopenia. Herein, the work in this thesis, assessed muscle quality through the quantification

of fat infiltration (myosteatosis), and markers of muscle contractility through specific force, pennation angle and pCSA. It was observed that IMAT within the quadriceps was greater in ESLD compared to controls. This is important as the presence of IMAT can negatively impact force production by disrupting muscle fibre alignments and result in reduced contractility and muscle function (463, 478, 479). Previous work has shown that muscle quality (measured by muscle attenuation from CT L3 SMI) has been shown to be an independent risk factor for mortality in those with ESLD (HR 9.12 (95% 2.9-29.0) (480), supporting its clinical significance. IMAT is also associated with metabolic dysregulation including insulin resistance, inflammation and alterations in myokines, which further impair muscle synthesis and drive proteolysis (463, 478, 479). Interestingly, we observed that those with HE and thereby hyperammonaemia, had a greater IMAT percentage than those without HE. There are several other potential mechanisms that contribute to the development of myosteatosis, in addition to hyperammonaemia and insulin resistance. These include mitochondrial dysfunction, reduced lipid storage capacity of subcutaneous adipose tissues and ageing related differentiation of muscle stem cells into adipocytes which should be considered in those with ESLD and those with HE (235, 298, 463). Furthermore, myosteatosis is not only associated with a poor prognosis, higher mortality and ESLD complications including HE, in those with cirrhosis(463), myosteatosis may also be associated with reduced survival post liver transplantation(481). IMAT has previously been poorly investigated in ESLD, however its importance is becoming increasingly recognised (463).

Muscle quality was also measured as specific force (defined as maximal knee extensor torque normalised to quadricep ACSA), however no difference was seen between ESLD and healthy controls. This would suggest that the ability of the contractile tissue in the quadriceps to

produce force is maintained in ESLD and the difference in force is due to a reduction in muscle mass. We also observed measures of muscle architecture including VL pennation angle, which influence muscle quality. The results demonstrated a reduction in the VL pennation angle; which can similarly be observed in age related sarcopenia (482, 483), and is thought to reflect a lower amount of contractile tissue along the aponeurosis, resulting in a potentially negative impact on force (484). PCSA accounts for muscle volume and muscle architecture (measured by pennation angle and fascicle length) therefore considers the ability of the muscle to produce and transmit force to the joint (485). A lower PCSA was observed in the ESLD group which may further elucidate the reduced force output aside from the muscle mass alone. Muscle architecture and quality was only measured in the vastus lateralis, therefore this does not represent the full quadriceps. Furthermore, whilst muscle quality in the truncal and upper limb can be explored, these results do indicate that alterations in muscle quality exist within the lower limb muscle group in those with ESLD.

In this study, there was a global reduction in measures of muscle function in those with ESLD compared to healthy controls. Chapter 3 describes the significant correlations between measures of upper and lower limb muscle mass and function. The loss of muscle mass and increase in IMAT, as described above can explain the reduced lower limb function observed. However, both upper and lower limb strength were weaker in those with ESLD with preservation of upper limb mass (via MAMC), suggesting that the decline in muscle strength appears global, irrespective of muscle group and mass. Interestingly the results did demonstrate a greater percentage difference in lower limb strength than upper limb strength, which further supports the notion that increased susceptibility of larger muscle groups such as the quadriceps when evaluating functional muscle loss (436, 439). As lower limb function

plays in important role in activities of daily living , there is a wider emphasis on the evaluation of these muscle groups.

HGS is a readily used measure of muscle function in those with ESLD with and has previously been shown to be strongly associated with mortality (197, 225, 355). However, it is important to recognised that measures of forearm and hand muscle mass, which are required for HGS, have not been investigated in ESLD. Based on clinical observations alone, proximal muscle wasting is a more frequently observed clinical phenotype (244). Studies in ageing cohorts have hypothesised there may be an association with HGS and forearm muscle thickness measured via ultrasound. This, however, remains poorly investigated and unsupported, with no current recommendations of forearms muscle measures in any society recommendation when evaluating sarcopenia or muscle loss (59, 486).

The composition measures of muscle strength and performance, SPPB and LFI, showed a similar trend to the above measures of muscle mass and strength, with reduced performance and lower scores in those with ESLD. Within the measures of SPPB, the wide range of chair stand time appeared to have a greater discriminating ability in all those with ESLD than the other measures. Complications of ESLD, such as ascites and HE, can influence the ability to perform functional tests such as chair stands(420). Longer chair stand times were observed between those with diuretic refractory ascites compared to controls, suggesting that ascites does restricting movement, potentially compounded by the additional fluid weight. Furthermore, in those with HE, both gait score and chair stand time were discriminatory when compared to controls. Chair stand score has been proven to be a strong predictor for waiting list mortality in those with ESLD (65), however there remain challenges in predictors of

functional measures in those with HE due to psychomotor dysfunction present (487). LFI, which incorporates balance, chair stand time and HGS, showed a greater pre-disposition to frailty in those with ESLD to controls. LFI incorporates both lower and upper limb functional measures providing a greater whole body assessment of muscle function. There was a significant correlation between LFI to total SPPB score as demonstrated in Chapter 3, suggesting that LFI, which was devised for those specifically with liver disease, may be a more comprehensive test for muscle function. Additionally, LFI is exempt from the ceiling or floor effect experienced with SPPB, allowing for a greater discrimination in studies (274, 488); thus, LFI may be a more comprehensive (sequential) test for muscle function than the SPPB, in patients with ESLD.

As part of the multi modal assessment, habitual daily physical activity was reduced in the ESLD cohort compared to controls. The results also showed a lower intensity gradient for those with ESLD; demonstrating that either less activity was accumulated at higher intensities, or that more activity was accumulated at lower intensities. However, in combination with the fact that reduced daily average intensity was observed in ELSD, it is likely that both aspects occurred. Physical activity is a key regulator of muscle mass (134) and as such these results highlight that reduced physical activity is likely an integral driver of muscle wasting in ESLD (276). This fact is then compounded by an increase in inactive time (a surrogate for sedentary time), in ESLD compared to controls. Inactivity strongly correlates with the development of both primary and secondary sarcopenia (134). The factors behind reduced physical activity and increased inactivity may include fatigue, reduced energy intake, daytime somnolence, and impaired cognition (489). It has previously been shown that an increase in moderate to vigorous physical activity to replace sedentary time and less intense physical activity was

associated with a reduction in sarcopenia (measured by DXA), and improved gait speed and HGS in older adults (490). To date, there have been no studies researching the effects of physical activity via accelerometery in the evaluation of sarcopenia or frailty in those with ESLD. However, there have been limited feasibility studies using step count targets as a marker of exercise for those with cirrhosis (276). Improved and optimised physical activity in those with ESLD may reduce the decline of their liver disease. A recent study of the UK biobank data found that an activity increase of an additional 2500 steps per day was associated with a 38% reduction in liver disease progression (360). Physical inactivity is also thought to be associated with other major chronic diseases. A cross sectional analysis of accelerometer measured physical activity in 96,000 UK biobank participants with chronic disease by Barker et al (491) identified that participants with any chronic disease engaged in 6% less moderate and 11% less vigorous activity per week, than those without a chronic disease. The chronic diseases in this study included cardiovascular, respiratory, gastrointestinal and genitourinary diseases and cancer, however it also included those with a mental health disorder. This highlight the association of inactivity in those with a chronic illness. In addition to associations with chronic disease, physical inactivity may be associated with a higher risk for cardiovascular disease. Ramakrishnan et al (492) investigated 90, 000 participants from the UK Bio Bank data who had 7 days of accelerometry data, without prior or concurrent cardiovascular disease. Physical activity is associated with lower risk for cardiovascular disease, with the greatest benefit got those who are activity are moderate and vigorous intensity levels. My study findings with respect to accelerometery measures, and other research described above, provides a foundation for further research investigating physical activity in ESLD, not only highlighting feasibility, but also highlighting key disease and

age groups to focus on. Furthermore, addressing co-factors such sedentary behaviour, mood and fatigue may further improve muscle mass and quality in ESLD (493, 494).

Hepatic encephalopathy remains an ongoing challenge in those with ESLD. It impacts nutritional intake, physical activity and comprehension of exercise, increased somnolence. It is important to recognised that all measures of function can be affected by HE (220). Even though there wasn't a significant difference between those with and without HE there was a trend towards HE having an impact on physical activity and overall ESLD had worse physical activity compared to controls in this study. There is a paucity of data within this field, most likely due to the ongoing challenge of psychological, cognitive and motor dysfunction that occurs with HE. Thus many with HE are excluded from studies given the variability in their ability to participate consistently (458). There is a prospective RCT in progress in Japan, investigating the physical activity on sarcopenia in those with overt HE, which is yet to be reported (495), however the research within this field is limited. My study results suggest that physical activity measures as measured by accelerometery, rather than arbitrary step counts, may be an early marker of muscle functional decline but this requires further validation.

Another complication of ESLD, ascites, can play an impactful role in the functional decline of patients with ESLD. It has been considered that those with ascites may have negatively impact lower limb performance and strength (420). Those with either diuretic controlled or diuretic refractory ascites did show a lower maximal peak knee extensor torque, but no difference demonstrated in dominant peak HGS, which may suggest there is a greater negative impact on lower limb muscle function. However, the most striking difference between those with ESLD with diuretic refractory ascites, ESLD with diuretic controlled ascites and ESLD with no

ascites fell between measures of physical activity. Those with diuretic refractory ascites had a lower overall habitual daily activity, a lower intensity of activity, and a significant lower average of time spent as a moderate to vigorous physical activity level highlighting the significant impact of ascites on daily activity. Diuretic refractory ascites is usually an advanced feature of chronic liver disease, and describes those who have renal dysfunction, which does not tolerate the use of diuretics to permit adequate diuresis (195). The ESLD with diuretic refractory ascites subgroup had a greater MELD and UKELD, therefore more advanced disease. It is known that those with a greater degree of disease severity, have a greater prevalence of muscle wasting (448), in agreement with these study findings of reduced lower limb maximal strength and physical function. Again, there is a paucity of data on physical activity in those with ESLD and ascites, however this study suggests that physical activity may represent early evidence of muscle functional change in those with ascites. Preventing muscle mass and function loss in those with ESLD remains a challenge which is undoubtedly impacted by the increased protein loss from frequency paracentesis, reduced appetite and early satiety from the subsequent compression effects (197). Further research into counteracting these factors especially with those with ascites is required.

The impact of the aetiology of liver disease was evaluated during this study. Those with ArLD demonstrated reduced lower limb function (reduced chair stand times, reduced maximal extensor peak torque), a greater LFI score, and worse physical activity measure (lower overall habitual daily activity and a great inactive time) compared to controls. The ArLD subgroup was a larger cohort in size compared to the other aetiologies which may explain some of the significant findings in this study. All participants were abstinent from alcohol, as a requirement either undergoing an assessment for LT +/- on the waiting list and managing

their disease itself. It is important to address the impact of alcohol (ethanol) on muscle wasting in this subgroup, despite their alcohol abstinence. Ethanol has been shown to accelerate muscle mass loss though an increased skeletal muscle autophagy and proteolysis (245). The long-term effect of those who remain abstinent from alcohol is less clear. It has been suggested that there may be significant degree of malnutrition from alcohol consumption that is difficult to improve following cessation of excess alcohol consumption, although this remains unproven (245). Alternatively, alcohol itself affects nutritional intake, particularly resulting in a lower protein consumption, higher fat percentage with micronutrient deficiencies, which further promote proteolysis (496). IMAT percentage was greater in ArLD than the other subgroups, which suggests that the muscle quality in those with ArLD is worse with a greater degree of myosteatosis. Fat infiltration within the muscle negatively impacts muscular contractility and function of the muscle itself (463, 478, 479). Whilst there were differences demonstrated muscle quality, the study did not demonstrate a significant difference in the measures of muscle mass in those with ArLD compared to controls which is likely due to the small sample size in each group.

Within the different aetiologies, muscle strength measures showed those with ArLD had a significant reduction in lower limb, extensor peak torque whereas those with NAFLD showed a significant reduction in upper limb strength, dominant peak HGS, compared to age and sex matched controls. There is little evidence regarding the effect of ESLD aetiology on the compartmental muscle loss. Compartmental lower limb muscle loss is well proven in those with age-related sarcopenia (436), however it remains unproven in secondary sarcopenia. HGS is the most frequently used measure of muscle strength in those with liver disease, however these results intimate that there should be a greater consideration of using lower

limb strength measure to risk stratify these patients. For future work, disease aetiology should be a consideration when evaluating muscle mass and functional measures as aetiology may manifest with differing severity of muscle compromise.

As previously described, disease severity in terms of UKELD and MELD does not consider nutritional indices of muscle mass or function measures. Our subgroup analyses of disease severity was defined by UKELD based on the national liver organ allocation system (473, 474). Despite this stratification of disease severity, the results only demonstrated a significantly lower quadriceps ACSA, and extensor peak torque in those with a UKELD of 50-55. One important factor in these results is the subgroup size; there were 29 of 42 participants within this group which will likely account for the significant findings for this subgroup alone. There may be some merit in further evaluating disease severity based on a clinical categorisation in the future with an adequate sample size in each severity group, which was in my initial methodology. Another element of the UKELD scoring system, that needs to considered is creatinine. Creatinine is lower in those with low muscle mass (497), therefore MELD and UKELD may not accurate recognised the impact of muscle wasting in these patients. Further research into a disease severity models with incorporates both muscle quantity and function (either performance or strength) may present a better predictive model for mortality and outcomes (211, 452, 498). It was not the aim of the current study and I felt that the sample size was not adequate to develop a predictive model for mortality in ESLD, however incorporating validated measures of both muscle mass and performance may improve liver disease severity risk stratification in the future.

The influence of ageing on muscle loss is well documented with the reduction in mass and strength in older adults (468). This phenomenon was reflected in our data. Those with ESLD aged between 60-71 had a lower quadriceps ACSA, alongside weaker knee extensor peak torque strength. This may support that those with ESLD have drivers of both primary and secondary sarcopenia influencing their muscle loss. Furthermore, those aged 50-59 and 60-71 years had reduced physical activity and lower sleep efficiency compared to controls. Older adults have demonstrated an increase in sedentary behaviour though their older years (499). This may suggest that those with a chronic disease may have a compounded effect of ageing, which manifests earlier than in primary sarcopenia alone. Lower limb muscle compartments can show reduced muscle mass and function with ageing (306, 436) therefore these results may support that within the above limitations.

Sleep plays an important role homeostasis and age-related alterations in sleep can be detrimental to muscle mass and function preservation (500). Ageing can alter normal circadian rhythms which can inhibit anabolic hormone cascades and enhancing catabolic pathways in skeletal muscle (501, 502). Some correlational studies have determined that reduced sleep duration or quality can lead to a loss of muscle mass (501, 502). Those with ESLD had a significantly reduced actual sleep time and sleep efficiency compared to controls, in this study. Whilst other studies have used subjective measures to quantify sleep (503), this is the first study to my knowledge that has used accelerometery to evaluate sleep parameters in those with ESLD. It provides objective evidence supporting altered circadian rhythm in those with ESLD, which is another potential driver for skeletal muscle catabolism. This is an important finding, as normal circadian rhythm and hormone balance is required for adequate muscle homeostasis (500). These findings do need further validation and research. Those

with ESLD aged 50-59 and 60-71 showed poor sleep efficiency compared to controls suggesting the impact of ageing in sleep on this cohort. Disturbances to sleep can have a detrimental effect on behaviour and quality of life in those with ESLD and is particularly compounded in those with HE (487, 504). Whilst there was no difference between sleep efficiency between those with ESLD with HE and ESLD without HE in this study, the influence of sleep alteration on quality of life, in particular compliance, fatigue and mood should be considered as it can affect muscle mass and performance (505, 506).

It is well known that sex related disparities in both muscle mass and body composition occur. (238, 471, 472) and our data further demonstrates this fact in ESLD for both muscle mass and strength. Men with ESLD had a greater quadricep peak ACSA, peak dominant HGS and knee extensor peak torque (data not shown) compared to females with ESLD. Females with ESLD had reduced chair stand times compared to control females, which was not demonstrated in ESLD males compared to their male controls. Females have a greater reduction in quadriceps mass from the 4th decade and this may account for the difference in lower limb performance, specifically chair stands affects and LFI which corporates this (238, 471, 472). Lower limb measures of performance may demonstrate an earlier change than conventionally used HGS in ESLD. There was a clear reduction in nearly all measures of lower limb muscle mass via MRI and ultrasound quadriceps in ESLD males compared to control males, which was not demonstrated in the females with ESLD compared to controls. Lower limb mass shows an early decline in primary sarcopenia which support the changes shown in this study (306, 436). Both upper and lower limb strength were reduced in ESLD males. Men have a greater proportion of upper body muscularity whoever both upper and lower limb mass compartments reduce over time/ with age (238, 471, 472). This pre-existing, greater

deposition of upper limb muscularity may provide a significant delta change in upper limb strength for males with ESLD. Further considerations for muscle mass and functional loss in males with ESLD is testosterone. Hypogonadism can be present in men with chronic liver disease. Testosterone targets androgen receptors and promotes muscle growth by triggering differentiation of satellite cells into new myocytes, and by also down regulating myostatin and upregulating IGF 1 promoting muscle synthesis (289). Lower testosterone is associated with men with ESLD with some studies suggesting that testosterone replacement may play a role in preventing sarcopenia (283). The role of sex hormones cannot be overlooked in muscle wasting. Chronic liver disease can cause menstruation disturbances in women (507). During menopause and ageing muscle mass and function decline occurs. Oestrogens can stimulate satellite cell proliferation and dampen the inflammatory stress damage on skeletal muscle (508). Furthermore, lack of oestrogens may promote mitochondrial dysfunction, cellular senescence and muscle proteolysis(508). Further studies are needed to evaluate the role of sex hormones in muscle wasting for those with ESLD.

Study limitations

There are limitations within this study. Due to SARS-CoV-2 pandemic, this study was paused from March 2020 until April 2021. Whilst all ESLD participants were recruited, this delayed the recruitment of our sex/age matched healthy control participants. Due to government and ethical restrictions in place, recruitment of the control cohort was limited to staff from the University of Birmingham and Queen Elizabeth Hospital Birmingham NHS Trust. Furthermore, due to the adapted hybrid model of working, with a greater emphasis on working from home mandates in place, it is possible that habitual daily activity, accessibility to usual physical

activities such as gyms, exercise clubs and dietary intake may have been negatively impacted in this control cohort.

In addition, there is a real need to understand how serial measures of muscle health perform and how human factors (disease severity, age, gender) impact on these over time in ESLD. The BRC sarcopenia themed project and I have undertaken serial measures of muscle mass, strength, function and quality on the current studies cohort both before and after LT. Even though analysing these serial measures was beyond the scope of the current PhD, the information will provide valuable insights into how muscle pathology evolves over a period of 3 months both before and after interventions (including LT).

4.5 Conclusion

In conclusion, the data demonstrate there is a clear reduction in muscle mass, quality and functional measures in those with ESLD. Differences in muscle mass were only seen in lower limb and not within truncal or upper limb indices, in those with ESLD, which was supported in the male ESLD group suggesting that compartmental muscle changes may occur in ESLD. Muscle strength was reduced in ESLD in both upper and lower limbs, with lower scores in the composite measures of function, compared to controls. There was a clear increase in myosteatosis in those with ESLD which may account for the changes in muscle function and performance. The study suggests that when evaluating changes in muscle composition, muscle mass, quality and function should all be considered.

The aforementioned data from this study demonstrates that muscle mass, function and performance is impacted by ascites, hepatic encephalopathy, aetiology of ESLD, age and sex.

One of the most important findings to highlight is altered physical activity in those with ESLD. This study suggest should be a greater consideration of both upper and lower limbs muscle mass and functional measures when evaluating muscle health in ESLD, in addition to considering variables such as disease aetiology, severity, age and sex.

5. Chapter Five: Identifying potential drivers of distinct sarcopenia phenotypes in End-stage liver disease (ESLD)

5.1 Introduction

Muscle wasting is a major concern in ESLD, with an increasing prevalence with advancing disease. It has also been established as an independent predictor for mortality and poor outcomes such as increased length of hospital admissions and ICU length of stay (256). Defining sarcopenia and frailty, a consequence of muscle wasting, is challenging in this patient population and criteria differ slightly to those set by EWGSOP (59). Furthermore, we recognise that these definitions of sarcopenia and frailty may not reflect the phenotypic heterogeneity of muscle wasting observed in these patients in clinical practice.

The phenotype characterisation of muscle wasting in those with ESLD currently relies on subjective clinical manifestations and assessment of muscle health, including: proximal muscle wasting on clinical examination, to measures of muscle mass and muscle function with reduced MAMC or HGS, respectively. There can be clear phenotypic differences in patients with ESLD, most apparent in those with complications of portal hypertension, as demonstrated by profound proximal muscle limb wasting, gross abdominal distension, yet a relatively preserved BMI in those with refractory ascites (71). The different aetiologies causing ESLD can also present differing body composition and phenotypes. For example, those with NAFLD who have a high prevalence of central obesity and ectopic fat deposition may have preserved proximal muscle mass on conventional measures of MAMC, however a significant proportion of mass will be attributed to a greater presence of myosteatosis (465). This can

result in what can be phrased as a 'muscle mass-to-function mismatch', in which they have good muscle mass but poor function. In contrast, clinical observations have highlighted that some patients with very poor muscle mass have preserved muscle function and functional independence (i.e. young age, sarcopenic patients with ArLD). Our understanding of why muscle mass-to-function mismatches occur in ESLD remains poor, and has potential ramifications of accurate patient selection for interventions (including transplantation) and targeted muscle (exercise) interventions in the future. One hypothesis for mass to function discrepancies is the quality of muscle, which may be determined by ectopic fat (myosteotosis), inflammatory state and regenerative capacity.

In addition to a paucity of research regarding the phenotypes of muscle wasting in those with ESLD, there is also inadequate consideration of the mechanistic drivers of the key muscle wasting phenotypes, thereby limiting progress in therapies in this field. It is well established that sarcopenia is due to an imbalance in muscle protein synthesis and breakdown, with a balance in favour of catabolism. Some of the established drivers for this in ESLD include: increased utilisation of amino acid substrates from skeletal muscle (262); a potential blunted anabolic response to amino acids (anabolic resistance) (239); insulin resistance reducing IGF-1 signalling and downregulation of the PI3K/Akt mTOR pathway; chronic inflammation (281) with activation of the NF$\kappa\beta$ pathway leading to activation of atrogenes, promoting proteolysis (281); mitochondrial dysfunction and reduced mitochondrial quantity (299); and potentially increased cellular senescence (509, 510). This supports the multifactorial causation of muscle wasting in ESLD and the need to delineate which of these factors drive the different potential phenotypes of sarcopenia and mass-to-function mismatches in ESLD.

The aim of this chapter is to define the clinical phenotypes of muscle wasting in ESLD and to analyse muscle mass-to-function mismatches. In doing so, using the healthy control cohort as a benchmark, I aimed to Identify potential mechanistic drivers of distinct sarcopenia phenotypes in ESLD by dividing the cohort into four distinct phenotypes, namely: (a) adequate mass, adequate function; (b) adequate mass, inadequate function; (c) inadequate mass, adequate function; and (d) inadequate mass; inadequate function.

5.2 Methods

5.2.1 Study population

Of the 53 participants with ESLD recruited to this study, 39 were included in the current analysis (i.e. complete dataset including MRI) alongside 18 age and sex-matched healthy control participants (see Chapter 3; Figure 3.1). In light of the positive findings with regards to lower limb assessment in ESLD, highlighted in chapters 3 and 4, the 39 participants were then sub-divided into the following distinct muscle phenotypes: Group 1 - adequate lower limb muscle mass and function, Group 2 - adequate lower limb muscle mass and inadequate lower limb function, Group 3 - inadequate lower limb muscle mass and adequate lower limb muscle function and Group 4 - inadequate lower limb muscle mass and function. The research modalities used and definitions of the phenotypic sub-groups are discussed below.

5.2.2 Muscle mass, strength and functional assessment modalities and methods

Chapter 2 provides detailed analysis of all muscle assessment modalities performed as well as an in-depth description of methodology. For purposes of accurate lower limb phenotyping (as highlighted in Chapter 3), I opted to utilise MRI quadricep volume index to define muscle mass and leg extensor peak torque via isokinetic dynamometry to define muscle function.

5.2.3 Identifying potential mechanistic drivers of distinct muscle phenotypes

Several methods were utilised to identify potential drivers of the four distinct muscle phenotypes in my cohort. These included muscle biopsy (transcriptomic analysis), serum cytokine, steroid and vitamin D analysis:

1. Vastus Lateralis muscle biopsy and transcriptomic analysis

Chapter 2 provided a detailed analysis of the vastus lateralis muscle biopsy protocol devised and used for this study. I led and performed the majority of the muscle biopsies included in this study. The transcriptomic analysis of muscle biopsy RNA was performed by Dr Sophie Allen and Dr Thomas Nicholson and as part of the National Institute for Health and Care Research Biomedical Research Centre Birmingham Sarcopenia collaborative.

Snap frozen muscle tissue was homogenised in TLR lysis buffer (74704, Qiagen, Manchester, UK), supplemented with 2-mercaptoethanol using a TissueRupter II (Qiagen). Next, total RNA was extracted using a RNeasy Fibrous Tissue Kit (74704, Qiagen) and treated with DNase (79254, Qiagen). Values of RNA integrity number were >7 (Aglient Bioanalyser). Library preparation and RNA-sequencing was performed using a QuantSeq 3' kit (Lexogen, Austria) and sequenced on an Illumina's NextSeq 500 by the Genomics Facility at the University of Birmingham. Once sequenced, RNA data was imported into Galaxy, a web platform (usegalaxy.org) and aligned to the human reference genome (hg38) with HISAT2 programming (511). Subsequently, binary alignment map (BAM) files were imported into Qlucore Omics Explorer v3.6 (Qlucore, AB, Lund, Sweden). Differential analysis was then performed following Trimmed Mean of M values normalization. Data was filtered to allow for the identification of differentially expressed genes using a multi-group statistical comparison

set at p <0.05. To establish differences between healthy controls and ESLD data, a two-group analysis was used with parameters set at P < 0.05 and a fold change > 1.5.

Differentially expressed genes from each patient cohort (P < 0.05, Fold Change > 1.5) were analysed using Ingenuity Pathway Analysis (IPA, Qiagen, UK) in order to determine the significance of the association with certain functions and pathways. To calculate the p-values of the association between pathways and genes a Fisher's exact test was used. To predict upstream regulators, p-values and z-scores were generated based upon the significant overlap present between the genes within the differentially expressed data set and known targets that are regulated by the transcriptional regulator.

2. Serum cytokine analysis

Bio-Rad Pro Human Cytokine 48 plex panel and Pro Human Diabetes 10 plex panels were used for the analysis of serum cytokines, following manufacturer' instructions. Briefly, 50µl of a 1X antibody bead stock for the cytokines was added to each well of the assay plate and the plate was washed twice with wash buffer. Serum samples were diluted 1:4 in sample diluent for the diabetes panel and used neat for the cytokine panel. Standard solution was added to the relevant wells and the plate incubated in the dark (60 minutes for Diabetes Panel, 30 minutes for Cytokine panel) at room temperature on an orbital mixer (speed 800-900 rpm; Grant Instruments Limited, Cambridge, UK). Post incubation, the plate was washed three times with wash buffer. 25µl of 1x detection antibodies was added to the wells and incubated in the dark for 30 minutes at room temperature on an orbital shaker (speed 800-900 rpm). The plate was then washed three times with wash buffer and 50µl of 1X streptavidin-RPE was added to the wells, followed by a 10-minute incubation at room temperature on an orbital shaker (speed

800-900 rpm) and three further washes with wash buffer. Cytokine concentrations were then analysed using a Luminex® 100TM instrument (Luminex® Corporation, Austin, Texas, USA).

Concentrations of Adiponectin, Myostatin and IGF-1 were quantified using the relevant ELISA DuoSet (DY008, DY788, DY291 R&D Systems, Minneapolis, MN, USA) following the manufacturer's protocol. For the adiponectin panels, a serum sample dilution of 1 in 15000 was used. For the IGF -1 and Myostatin, serum was used neat. Briefly, capture antibody was diluted in PBS (pH 7.2) and added to the required wells of an uncoated nunc-ImmunoT ELISA plate (Thermo Fisher Scientific, MA, USA) overnight at room temperature. For the Myostatin panel, a reagent activation step was required where 62.5µl of serum was added to 12.5 µl of HCl. Following a 10-minute incubation at room temperature 12.5 µl of 1.2M NaOH/0.5 M HEPES was added, mixed and then further 400 µl of reagent diluent was add. The following day, wells were washed 3 times with 300 µl wash buffer (PBS pH 7.2, containing 0.05% Tween® 20) and then blocked with reagent diluent (PBS containing 1% BSA) for 1 h. Plates were then aspirated and washed as described above. Next, protein standards were serially diluted 2-fold in reagent diluent. Standards and samples were then added to the plate in duplicate and incubated for 2 h at room temperature.

Following incubation, plates were again washed as above, before the addition of capture antibody for 2h at room temperature. Streptavidin-HRP was then added to wells for 20 min at room temperature in the dark. Following a final wash step substrate solution was added to wells for up to 20 min and reactions were stopped by the addition of stop solution (2N H_2SO_4) and optical density measured at 450 nm using a BioTek EL808 microtiter plate reader (BioTek, Swindon, UK). The average absorbance for each standard was used to generate a standard

curve using GraphPad Prism version 9 statistical package. Concentrations of serum samples were then calculated using this standard curve. For any values that were below the lower limit of quantification, a midpoint value between 0 and the lower limit of quantification was used.

3. Steroid and vitamin D analysis

Steroid and Vitamin D analysis was conducted by mass spectrometry the Steroid Metabolome Analysis Core, Institute of Metabolism and Systems Research, University of Birmingham. Steroid internal standards were purchased from Steraloids UK, Sigma Aldrich UK, Toronto Research Chemicals, Canada or Cambridge Isotope Laboratories, UK. Steroids quantified were aldosterone, cortisol, cortisone, 11-deoxycortisol, corticosterone, 11hydroxyandrosterone, 11ketoandrostenedione, 11hydroxytestosterone, 11ketotestosterone, androstenedione, testosterone, Dehydroepiandrosterone (DHEA), Dihydrotestosterone, deoxycorticosterone, 17hydroxyprogestrone, progesterone and Dehydroepiandrosterone Sulphate (DHEAS). Vitamin D metabolites quantified were 25-hydroxyvitamin D_3 (25OHD3) , 25-hydroxyvitamin D_2 (25OHD2), 3-epi-25-hydroxy Vitamin D3 (3-epi-25OHD3) and 24,25-dihydroxyvitamin D3 (24,25(OH)) (512-516).

Individual stocks for each authentic reference steroid were prepared in methanol at concentration of (1mg/ml) and stored at -80°C. These stocks were combined and diluted to prepare a calibration series in phosphate buffered saline with 0.1% bovine serum albumin.
Each steroid was quantified relative to a calibration series made of a matrix spiked with authentic reference materials ranging from 0.02 to 250ng/mL (with inclusion of a blank) and an appropriate internal standard. After addition of an internal standard mixture steroids were extracted from 200µL of serum via liquid/liquid extraction using 1mL of MTBE (tert-butyl

methyl ether). This organic layer was removed, evaporated to dryness under nitrogen at 55°C. Samples were then reconstituted in 150µL of 50/50 methanol/water and run by LC-MS/MS (liquid chromatography-mass spectrometry).

For DHEAS a calibration series from 250 to 10000 ng/mL was prepared in the same matrix. DHEAS was extracted from 20µL of serum after addition of internal standard. 100µL of acetonitrile was added to the sample and proteins were precipitated with 100µL of $ZnSO_4$. The samples were spun and the top 100µL removed and evaporated under nitrogen at 55°C. The dried extract was reconstituted in 200µL of 50/50 methanol/water and run by LC-MS/MS.

For Vitamin D, a calibration series from 0.063 to 128ng/mL (with inclusion of a blank) was prepared in 50:50 methanol: water solution. After addition of an internal standard mixture Vitamin D metabolites were extracted from 200µL of serum. Firstly, samples underwent protein precipitation by the addition of 80µL of methanol, 50µL of isopropanol and 100µL of water. The samples were then vortexed, spun by centrifugation and the supernatant transferred on to a supported liquid–liquid extraction plate (SLE). Vacuum was applied to the plate to ensure absorption of the sample into the SLE sorbent and Vitamin D metabolites extracted by two application of MTBE/ethyl acetate solution (90/10%). The eluent was evaporated under nitrogen at 50°C. Samples were reconstituted in 200µL of 50/50 methanol/water and run by LC-MS/MS.

These methods were previously optimised and validated by the Steroid Metabolome Analysis Core (14). A Waters-Xevo XS mass spectrometer with an Acquity uPLC system was used for quantification of all analytes. Serum steroids were separated using a methanol/water

gradient system on a Phenomenex Luna column at 60°C, with post column infusion of NH_4F as an ionisation additive. DHEAS was quantified using a methanol/water gradient system with 0.1% formic acid on a Waters HSS T3 column at 45°C. Vitamin D metabolites were separated using a methanol/water gradient system with 0.1% formic acid on a Waters UPLC BEH Phenyl at 40°C

5.2.4 Statistical analysis

All data were analysed using GraphPad Prism software, version 9 (La Jolla, CA, USA). The data were evaluated for normality with D'Agostino and Pearson test. All normally distributed data are presented as mean ± standard deviation and as median and IQR when non-normally distributed. The remainder of the analysis is divided into two phases:

Phase 1: To investigate if there are any differences in clinical parameters and muscle biology between the four distinct muscle phenotypes (mass-to-function mismatches) in ESLD:

Using the data from the age sex matched healthy controls, sex specific 25th quartile threshold was established for measure of muscle mass (MR quadricep volume index) and muscle function (leg extensor peak torque) (Table 5.1). Those with a quadriceps volume index of less than or equal to 25th quartile were defined as **'inadequate muscle mass'**. Those with a volume index greater than 25th quartile were defined as having **'adequate muscle mass'**. Those with a leg extensor peak torque less than or equal to 25th quartile were defined as **'inadequate muscle function'**. Those with a peak torque of greater than 25th quartile were defined as having **'adequate muscle function'**. The cohort was divided into four key domains based on adequate or inadequate quadriceps volume index (muscle mass) and knee extensor peak torque (muscle function) (Figure 5.1).

The following analyses were performed to compare clinical, muscle and biological parameters between the 4 distinct groups of muscle mass and function. This was analysed using several methods including unpaired two tailed t-tests and a one-way ANOVA for normally distributed data; and a Mann Whitney U test or Kruskal Wallis ANOVA for non-parametric distributed data. The level of significance for all tests was set at $p<0.05$.

Table 5-1: Sex specific values for inadequate lower limb muscle mass and function

	Controls	**Absolute values (≤25% centile)**
Lower limb mass	**Volume index (cm3/m2)**	≤295.9
	Male	≤375.6
	Female	≤270.2
Lower limb strength	**Extensor peak torque (Nm)**	≤164.7
	Male	≤137.8
	Female	≤89.8

Phase 2: To investigate if there are any distinct clusters of muscle parameter and biological variables within the ESLD cohort, healthy volunteers and defined muscle sub-groups:

A principal component analysis was performed on all the variables of muscle mass strength and performance in addition to the cytokines and steroid variables measures, as described in Table 5.2. For any missing values for the cytokine and steroid data analysis, the mean for that specific variable was used as a substitute. Principal components describe the linear combinations of the original data variables. The principal component 1 (PC1) explains the maximum variance and subsequent principal component factors explain progressively smaller portions of the total variance. Principal component plots and loading plots were created from the analysis. The principal component plots show a data plot for each of the participants used in the analysis. The loading plots show the linear coefficients for the principal components.

Table 5-2: Analytes applied to Principle Component Analysis (PCA)

Domain	Assessment modality	Variable measures
Mass	Ultrasound	Vastus lateralis muscle thickness (cm)
	MRI	Quadricep Cone volume (cm^3)
		Quadricep Volume index (cm^3/m^2)
		L3 skeletal muscle index* (cm^3/m^2)
	Anthropometry	Mid arm muscle circumference (cm)
	Bio impedance analysis	Fat free mass (kg)
Quality	MRI	Quadricep IMAT (%)
	MRI Isokinetic dynamometry	Specific force (Nm/cm^2)
Architecture	Ultrasound	Vastus lateralis fascicle length (cm) Vastus lateralis Pennation angle
Strength	Isokinetic dynamometry	Knee extensor peak torque (Nm) Knee extensor peak torque to body weight (Nm) Knee extensor average power (watts)
	Anthropometry	Handgrip strength (kg) (dominant and non-dominant, peak and average)
Function	Composite tests	Short physical performance battery (total, gait, balance and chair stand score, chair stand time) Liver frailty index
	Accelerometry	Overall daily activity (mg) Intensity gradient Total MVPA (>100mg) (minutes/day) Total inactive time (mins) Actual sleep time (hours) Sleep efficiency (%)
Cytokine assays	ELISA Luminex	FGF basic, Eotaxin, G-CSF, GM-CSF, IFN-γ, IL-1β, IL-1ra, IL-1α, IL-2Rα, IL-3, IL-12 (p40), IL-16, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, GRO-α, HGF, IFN-α2, LIF, MCP-3, IL-10, IL-12 (p70), IL-13, IL-15, IL-17A, IP-10,MCP-1 (MCAF), MIG, β-NGF, SCF, SCGF-β, SDF-1α, MIP-1α, MIP-1β, PDGF-BB, RANTES, TNF-α, VEGF, CTACK, MIF, TRAIL, IL-18, M-CSF, TNF-β C-peptide, ghrelin, GIP, GLP-1, Glucagon, insulin, leptin, PAI-1 (total), Resistin, visfatin Myostatin
Vitamin D metabolites	Mass spectrometry	25OHD3 Total (25OHD3+ 25OHD2), 25OHD3, 25OHD2, 3-Epi-25OHD3, 24,25(OH)2D3
Steroid hormones	Mass spectrometry	Aldosterone, progesterone, Testosterone, cortisone, cortisol, cortisol: cortisone ratio, DHEA, DHEAS, cortisol: DHEA S ratio

Fibroblast growth factor (FGF basic), Granulocyte colony-stimulating factor (G-CSF), Granulocyte-macrophage colony-stimulating factor (GM-CSF), Interferon gamma (IFN-γ), interleukin-1 beta (IL-1β), interleukin-1 receptor antagonist (IL-1ra), interleukin-1 alpha (IL-1α), interleukin-2 receptor alpha (IL-2Rα), interleukin-3 (IL-3), interleukin-12 (IL-12), interleukin-16 (IL-16), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-9 (IL-9), growth regulated alpha (GRO-α), hepatocyte growth factor (HGF), interferon alpha 2 (IFN-α2), leukaemia inhibitory factor (*LIF*),Monocyte chemotactic protein-3 (MCP-3), interleukin-10 (IL-10), interleukin-13 (IL-13), interleukin-15 (IL-15), interleukin-17a (IL-17a), Interferon gamma-induced protein 10 (*IP-10*), *Monocyte chemoattractant protein-1* (*MCP-1),* monokine induced by interferon-gamma (MIG) nerve growth factor (*β-NGF,* Stem cell factor

(*SCF,* Stem cell growth factor-beta (*SCGF-β) stromal cell-derived factor 1* (*SDF-1* α*)* Macrophage Inflammatory Proteins (MIP 1α) , Macrophage Inflammatory Proteins (MIP 1*β), Platelet-Derived Growth Factor-BB (PDGF-BB),* (Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted (RANTES), Tumour necrosis factor alpha (TNF-α) Vascular endothelial growth factor (VEGF) CC chemokine, cutaneous T cell-attracting chemokine (*CTACK),* macrophage migration inhibitory factor (*MIF), t*umour necrosis factor (TNF)-related apoptosis-inducing ligand (*TRAIL)* macrophage colony-stimulating factor (*M-CSF*), Tumour necrosis factor beta (TNF- *β*)interleukin 18 (IL-18), Glucose-dependent insulinotropic polypeptide (GIP), Glucagon-like peptide-1 (*GLP-1*) agonists, p*lasminogen activator inhibitor*-1 (*PAI-1*),

5.3 Results

5.3.1 Baseline characteristics

In total, 39 participants with ESLD and 18 controls were included in the current analysis. The ESLD cohort was age and sex matched to 18 healthy control participants with a mean age of 50.0±15.2 years (11 males/7 female) with a mean BMI of 25.1 [4.3]. The clinical demographics and muscle parameters of these participants are described in chapters 3 and 4.

5.3.2 Phase 1: Distinct muscle phenotypes (mass-to-function mismatch) of ESLD cohort

Of the 39 participants with ESLD, 9 (23.1%) had both adequate lower limb muscle mass and muscle function, 13 (33.3%) had adequate lower limb muscle yet inadequate lower limb muscle function and 17 (43.6%) had inadequate lower limb muscle mass and function. None of the patients met the criteria for inadequate lower limb muscle mass and adequate lower limb function. Therefore, the group 2 had a mismatch in mass-to-function, whereas group 1 and 4 had either adequate mass/function or inadequate mass/function, respectively.

Adequate lower limb muscle mass Adequate lower limb muscle function (Group 1) 9 (23.1%)	Adequate lower limb muscle mass Inadequate lower limb muscle function (Group 2) 13 (33.3%)
Inadequate lower limb muscle mass Adequate lower limb muscle function (Group 3) 0 (0.0%)	Inadequate lower limb muscle mass Inadequate lower limb muscle function (Group 4) 17 (46.2%)

Figure 5-1: Categorisations of the ESLD cohort as per adequate and inadequate sex specific muscle mass and function criterion.

The mean age in was significantly higher in patients with inadequate muscle function (Group 2 58.0 ± 9.6 years; Group 4 56.5 ± 9.5 years) than those with preserved muscle mass and function (Group 1). The group (4) with co-existing inadequate muscle mass and function was male predominant (76.4%), whereas the other groups had gender equality. The predominant aetiology of ESLD for each of the muscle phenotype groups was ArLD, with similar mean MELD and UKELD score (Table 5.3) throughout. There were no differences in the rates of hepatic encephalopathy (all on rifaximin) between the distinct muscle phenotypes. There were high rates of portal hypertension in all muscle phenotype groups, however, the rates of ascites and SBP were lower in those patients with both adequate muscle mass and function (Group 1). Interestingly, those with inadequate function (Group 2 and 4) had higher rates of overall mortality than those with both adequate muscle mass and function (Group 1).

Table 5-3: Baseline demographic and biochemical parameters for each phenotype of ESLD and healthy controls cohorts. Note: Group 3 not included in the table as no participants met this criteria of inadequate mass and adequate function.

	Adequate mass Adequate function **Group 1**	Adequate mass Inadequate function **Group 2**	Inadequate mass Inadequate function **Group 4**	P value
Total (n)	9	13	17	
Age (years)	47.9 [11.5]	58.0 [9.6]	56.5 [9.5]	0.06
Gender (male : female)	4:5	6:7	13:4	-
BMI	37.8 [5.4]	30.7 [7.7]	27.1 [4.9]	<0.05
DISEASE SPECIFIC				
MELD	14.3 ± 5.1	13.9 ± 5.3	13.1 ± 4.2	0.81
UKELD	53.0 ± 3.1	52.5 ± 2.9	52.9 ± 3.6	0.86
Aetiology	ArLD 44.4%, NAFLD 33.3% Immune mediated 22.2%	ArLD 46.2%, Immune mediated 46.2%, NAFLD 7.7%	ArLD 53.0%, Immune mediated 29.4%, NAFLD 5.9%, other 11.8%	-
Hepatic encephalopathy	6 (46.2)	7 (53.8)	8 (47.1)	0.65
Portal Hypertension	9 (100.0)	11 (84.6)	15 (88.2)	0.51
Ascites	5 (55.6)	10 (80.0)	15(88.2)	0.13
- Diuretics	5 (55.6)	9 (69.2)	9 (52.9)	0.41
- LVP	0	1 (7.6)	6 (35.2)	<0.05
- SBP	1 (11.1)	3 (23.1)	5 (29.4)	0.59
- Antibiotic prophylaxis	1 (11.1)	3 (23.1)	5 (29.4)	0.59
Varices	6 (46.2)	7 (53.8)	10 (58.8)	0.85
- Primary prophylaxis	4 (44.4)	4 (30.8)	7 (41.2)	0.79
LT	6 (46.2)	8 (61.5)	11 (64.7)	0.97
Outcome deceased	0	2 (15.4)	2 (11.8)	0.51
BIOCHEMICAL PARAMETERS				
Haemoglobulin (g/dL)	119.2 [23.4]	129.1 [9.3]	129.5 [15.8]	0.27
White cell count (10^9/L)	5.0 [2.2]	4.6 [2.1]	5.0 [1.5]	0.83
Neutrophils (10^9/L)	3.0 [1.5]	2.7 [1.5]	3.0 [1.0]	0.85
Platelets (10^9/L)	88.4 [19.5]	119.0 [79.2]	125.4 [76.4]	0.89
INR	1.3 [0.3]	1.4 [0.6]	1.2 [0.2]	0.37
Bilirubin (µmol/L)	48.2 [42.7]	45.5 [29.5]	39.5 [21.7]	0.75
ALT(IU/L)	35.4 [26.0]	66.6 [72.5]	52.7 [60.2]	0.49
ALP (IU/L)	204.1 [154.1]	219.6 [137.4]	303.4 [419.8]	0.65
Albumin (g/L)	33.4 [8.3]	34.2 [4.8]	36.7 [4.9]	0.32
Sodium (mmol/L)	137.3 [4.2]	138.0 [3.0]	136.2 [3.6]	0.38
Urea (mmol/L)	5.7 [3.9]	5.7 [2.3]	8.7 [7.0]	0.21
Creatinine(µmol/L)	84.6 [34.9]	66.8 [17.6]	88.4 [36.5]	0.14
Ammonia(mg/ml)	67.9 [31.1]	92.3 [61.4]	65.2 [22.0]	0.22

*Data expressed as mean [standard deviation], a direct percentage or an absolute value with (percentage %)

The mean values for all the measures of mass and functional measures are described below in Table 5.4. Of note, the groups were pre-defined based on MRI quadricep volume index

(mass) and dynamometry quadricep extensor peak torque (function), therefore the differences in these parameters between groups 1, 2 and 4 are to be expected. Interestingly, though there was no striking difference in myosteatosis (IMAT%) between the groups.

Table 5-4: Muscle mass and function variables for each distinct muscle phenotype group and healthy controls.

	Adequate mass Adequate function Group 1	Adequate mass Inadequate function Group 2	Inadequate mass Inadequate function Group 4	Healthy control cohort	P value
Total (n)	9	13	17	18	
MUSCLE MASS MEASURES					
MRI quadricep Volume index (cm^3/m^2)	431.7 [53.2]	353.3 [62.6]	301.3 [39.4]	409.3 [107.0]	<0.0001
MRI Cone volume (cm^3)	1319 [258.1]	1041 [230.4]	923.9 [177.7]	1227 [393.5]	<0.01
MRI Peak quad ACSA (cm^2)	65.8 [10.8]	52.2 [11.9]	48.1 [7.6]	64.8 [17.8]	<0.001
MRI L3 SMI (cm^3/m^2)	49.0 [4.3]	40.5 [10.6]	37.3 [8.0]	43.7 [8.7]	<0.05
USS VL Muscle thickness (cm)	2.4 [0.3]	2.2 [0.3]	1.9 [0.4]	2.4 [0.5]	<0.01
Bio impedance Fat free mass (kg)	68.0 [14.5]	59.5 [12.3]	59.3 [11.2]	51.0 [11.5]	<0.05
MAMC (cm)	31.1 [6.8]	26.8 [6.3]	26.5 [4.6]	26.5 [4.4]	0.16
MUSCLE QUALITY MEASURES					
Specific force (Nm/cm^2)	2.3 [0.4]	1.7 [0.4]	1.8 [0.7]	2.2 [0.5]	0.10
MRI IMAT (%)	10.2 [3.2]	12.1 [4.2]	9.8 [2.9]	5.2 [1.8]	<0.0001
MUSCLE FUNCTION MEASURES					
Knee extensor peak torque (Nm)	135.1 [31.6]	88.5 [28.4]	89.1 31.8]	142.5 [51.0]	<0.001
Knee extensor average power (watts)	77.8 [23.2]	49.5 [17.3]	51.4 [20.8]	84.7 [31.6]	<0.001
SPPB total (max 12)	10.7 [1.7]	9.4 [2.7]	10.4 [2.3]	12 [0.0]	<0.01
Chair stand time (secs)	11.1 [4.0]	12.5 [4.2]	12.6]5.8]	7.6 [5.8-7.7]	<0.01
LFI (a.u.)	3.4 [0.6]	3.6 [0.4]	3.6]0.5]	2.8 [0.6]	<0.0001
Dominant peak HGS (kg)	32.9 [8.4]	31.4 [8.2]	33.3 [7.8]	39.4 [10.7]	0.06
Non-dominant peak HGS (kg)	32.8 [8.3]	29.1 [8.5]	29.9 [6.9]	38.2 [8.5]	<0.01
Total inactive time (mins)	757.1 [143.0]	822.6 [101.9]	753.9]158.4]	710.4 [124.9]	0.08
Overall daily activity (mg)	21.5 [8.5]	17.1 [5.8]	18.3]8.1]	29.2 [8.9]	<0.001
Sleep time (hours)	5.9 [0.5]	5.9 [1.6]	5.9]1.6]	7.1 [0.8]	<0.0001
Sleep efficiency (%)	0.8 [0.1]	0.8 [0.04]	0.8 [0.1]	87.1 [6.8]	<0.001

*Data expressed as mean [standard deviation]

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Table 5.4 describes the mean for selected cytokine, vitamin D and steroid analytes for each distinct group and healthy controls. Those with adequate muscle mass and function (group 1) has a significantly lower cortisol: cortisone ratio than those with inadequate function and

controls. There was no significant differences observed with pro and anti-inflammatory cytokines between the groups.

Table 5-5: Selected cytokines, vitamin D and steroid analytes for each distinct muscle phenotype group and healthy controls.

	Adequate mass Adequate function Group 1	Adequate mass Inadequate function Group 2	Inadequate mass Inadequate function Group 4	Healthy control cohort	P value
Total (n)	9	13	17	18	
STEROID AND VITAMIN D ANALYTES					
Cortisol (nM)	255.2 [98.0]	268.5 [91.8]	298.6 [90.6]	332.8 [96.6]	0.15
DHEAS (nM)	662.3 [590.2]	702.1 [1052]	614.0 [697.4]	513.0 [290.0]	<0.05
Cortisol: cortisone ratio	4.8 [1.7]	5.4 [3.0]	5.4 [2.2]	7.4 [2.4]	<0.05
Cortisol: DHEAS ratio	0.5 [0.3]	0.7 [0.3]	1.0 [0.6]	0.1 [0.0]	<0.0001
Total 25 OH D3 (nmol/L)	36.0 [24.4]	43.6 [20.5]	39.8 [17.9]	48.2 [16.0]	0.43
CYTOKINES					
IL-1 (pg/ml)	4.2 [3.0]	11.9 [12.1]	6.8 [9.9]	5.0 [3.9]	0.14
IL-16 (pg/ml)	30.3 [50.4]	16.9 [10.0]	12.6 [11.3]	6.0 [3.9]	0.05
IL-17 (pg/ml)	2.8 [1.6]	4.5 [5.3]	2.9 [1.6]	3.2 [2.7]	0.50
IFN χ (pg/ml)	0.5 [0.4]	0.9 [0.6]	0.9 [1.2]	2.6 [7.1]	0.51
TNF α (pg/ml)	3.9 [3.2]	4.2 [3.5]	3.0 [2.3]	2.5 [1.8]	0.30
Myostatin (pg/ml)	1261 [601.6]	1337 [548.4]	1184 [757.2]	1797 [1145]	0.16
IL-1 receptor antagonist (pg/ml)	0.82 [0.53]	3.4 [8.8]	0.7 [0.8]	1.8 [3.3]	0.41
IL-10 (pg/ml)	112.3 [66.2]	99.4 [45.7]	88.6 [53.3]	156.2 [128.6]	0.11

*Data expressed as mean [standard deviation]

There were and 17/18 healthy control participants and 23/39 patients with ESLD ((Group 1 (7/23), Group 2 (5/23) and Group 4 (11/23)) who had a vastus lateralis muscle biopsy performed, with adequate sampling obtained and analysed for the transcriptomic analysis.

5.3.3 Phase 2: Principal component analysis (PCA) of clinical, muscle and biological parameters in ESLD

A PCA was performed on all the data described above. Figure 5.2 shows the principal component plot outlines of all the measured variables (muscle mass, muscle functional measures, cytokines, vitamin D and steroid hormones) for the patients with ESLD and the healthy controls and the principal components. The figure shows that there was no distinct separation of the data into any definite clusters, either for the total ESLD cohort or within the distinct muscle phenotypes for ESLD.

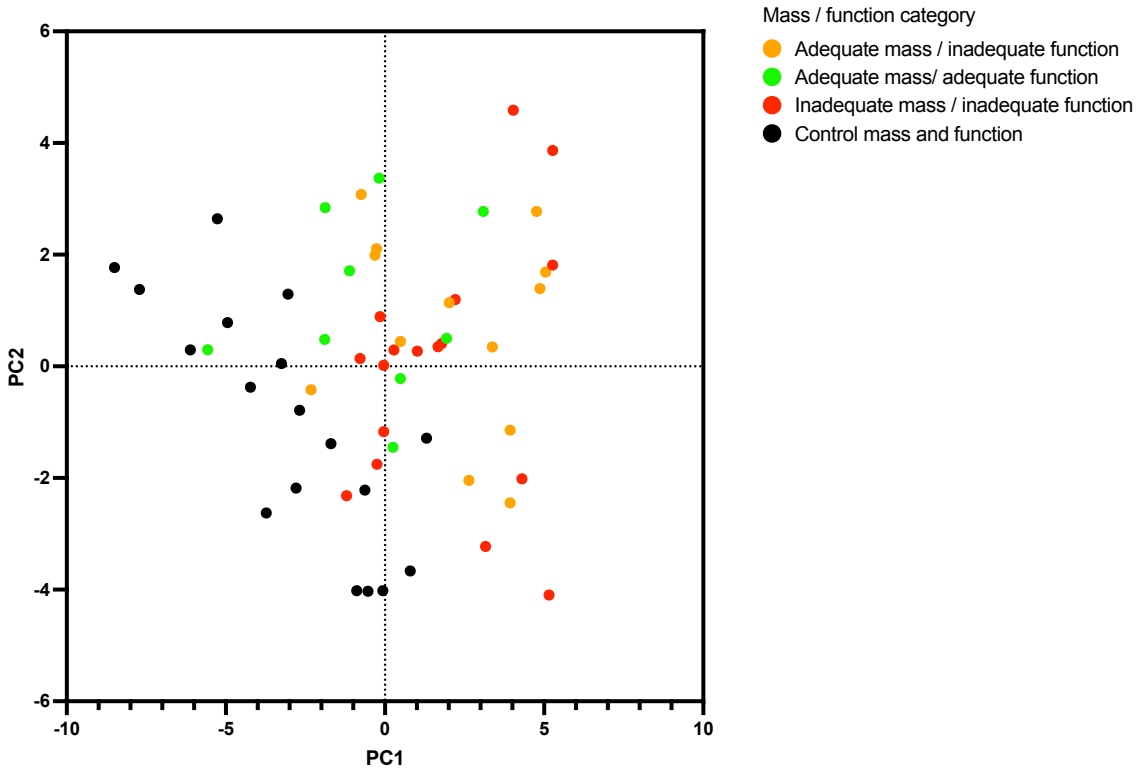

Figure 5-2: Principal component scores for all measures of muscle mass and function, and total cytokine, steroid hormone and vitamin D profiles for phenotype of ESLD and healthy controls.

(The healthy controls are classified in black, Group 1 (Adequate mass and function) are classified in red, Group 2 (adequate mass with inadequate function) are classified in orange, with Groups 4 (inadequate mass and function) classified in red)).

Figure 5.3 shows a principal component plot which outlines the projection of measured muscle variables (muscle mass, muscle functional measures) only, for the patients with ESLD and the healthy controls. The figure shows that there was no separation of the cohorts (ESLD and controls) with this specific selection of muscle variables.

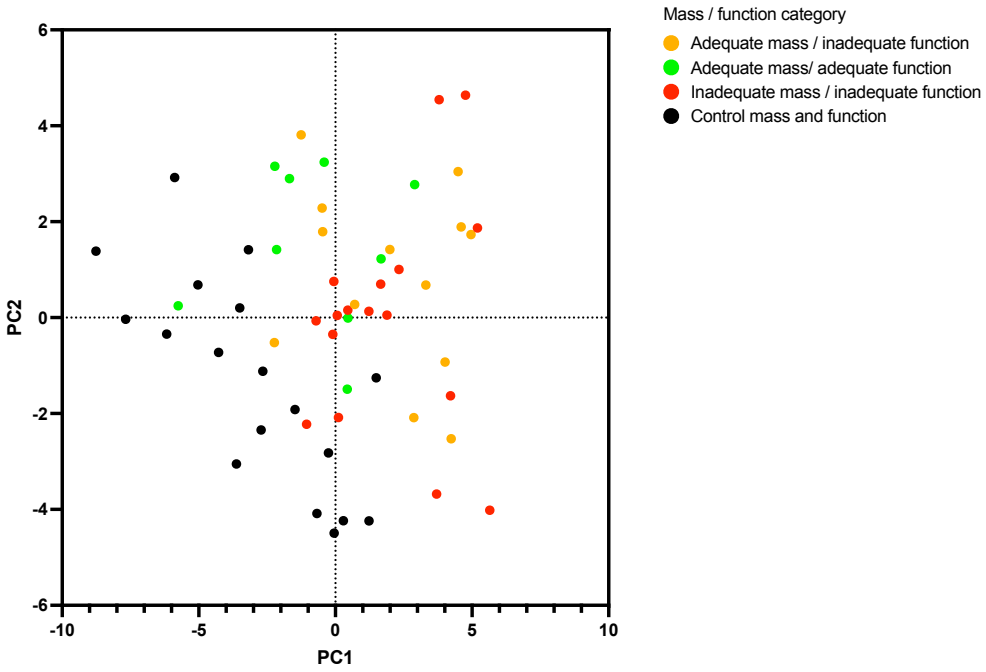

Figure 5-3: Principal components (PC) scores for all measures of muscle mass and function for each phenotype of ESLD and healthy controls

(The healthy controls are classified in black, Group 1 (Adequate mass and function) are classified in red, Group 2 (adequate mass with inadequate function) are classified in orange, with Groups 4 (inadequate mass and function) classified in red)).

Figure 5.4 shows the principal component plot that outlines the projection of the measured variables (cytokines, vitamin D and steroid hormones) only for the patients with ESLD and the healthy controls. This figure shows that the first principal component (PC1) did separate a cluster of the healthy control cohort and Group 4 (inadequate mass and function). Furthermore, from the loading plot (Figure 5.5) Group 4 appear to be separated by loadings from IFN beta, cortisol:DHEA ratio whereas the healthy control group appear to be separated

by loadings from DHEAS and the cortisol to cortisone ratio. None of the principal component plots showed any outliers in the data.

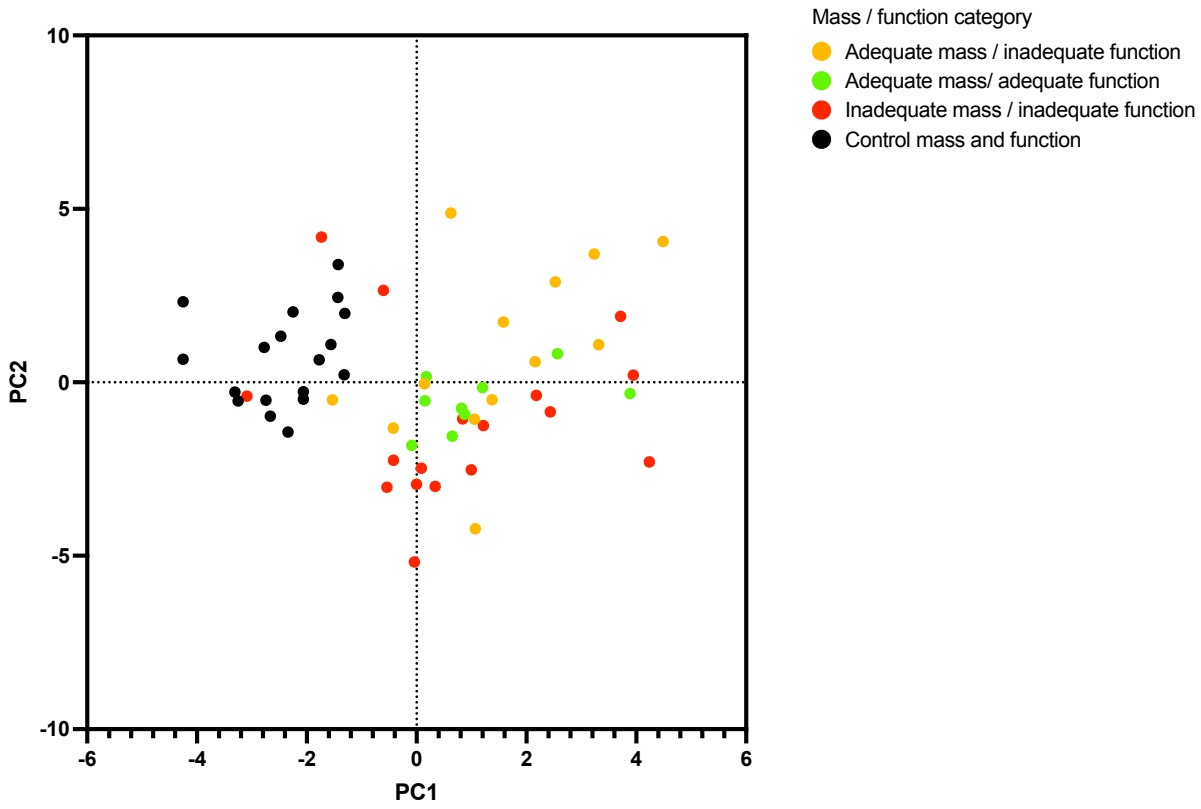

Figure 5-4: Principal component (PC) scores for cytokine, steroid hormone and Vitamin D variables for each phenotype of ESLD and healthy controls

(The healthy controls are classified in black, Group 1 (Adequate mass and function) are classified in red, Group 2 (adequate mass with inadequate function) are classified in orange, with Groups 4 (inadequate mass and function) classified in red)).

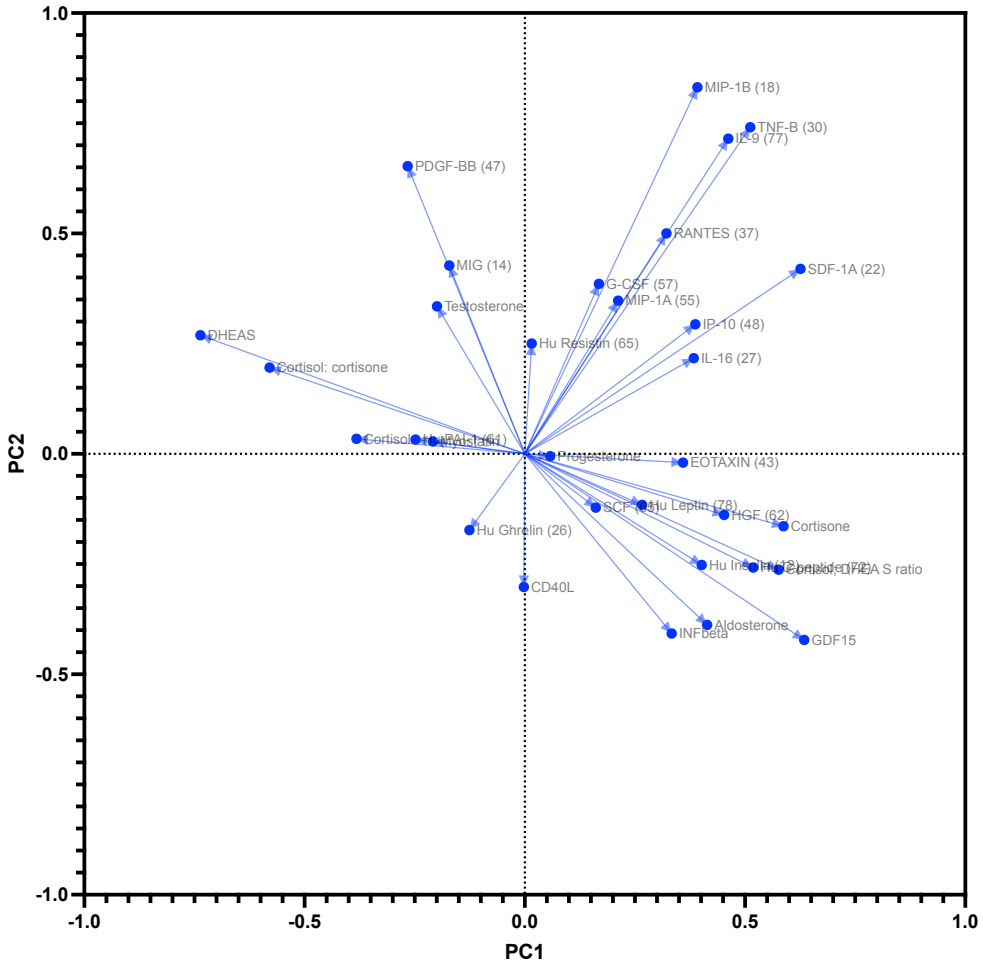

Figure 5-5: Loading plot for cytokine, steroid hormone and Vitamin D profiles of ESLD and healthy controls

Figures 5.6, 5.7 and 5.8 depict canonical pathway and network analysis for the transcriptomic data in relation to the clinically determined phenotype groups of ESLD (1,2,4).

There were some distinct pathways which were enriched in some of the comparisons. In the comparison of Group 2 (adequate muscle mass, inadequate muscle function) with Group 1 (adequate muscle mass and function) a variety of pathways were altered notably those concerned with differentiation (retinol biosynthesis, RXR function, FXR/RXR activation) as well as coagulation pathways (Figure 5.6) suggesting in impact on muscle function. Other

pathways such as senescence, p70S6K signalling and mTOR were observed in Group 4 compared to 2 (Figure 5.7), suggesting muscle ageing and anabolic resistance may predominate here. There was a clear predominance of proinflammatory pathways such as mitochondrial dysfunction and oxidative phosphorylation and protein synthesis pathways such an eIF2 signalling, as well as pro-fibrotic pathways observed when comparing Group 1 (adequate muscle mass and function) and Group 4 (inadequate muscle mass and function). Therefore, suggesting that these pathway are enriched in Group 4 (Figure 5.8). There are proinflammatory pathway observed in the comparison of Group 3 to Group 1 with LPS/IL1 mediated inhibition of RXR function present.

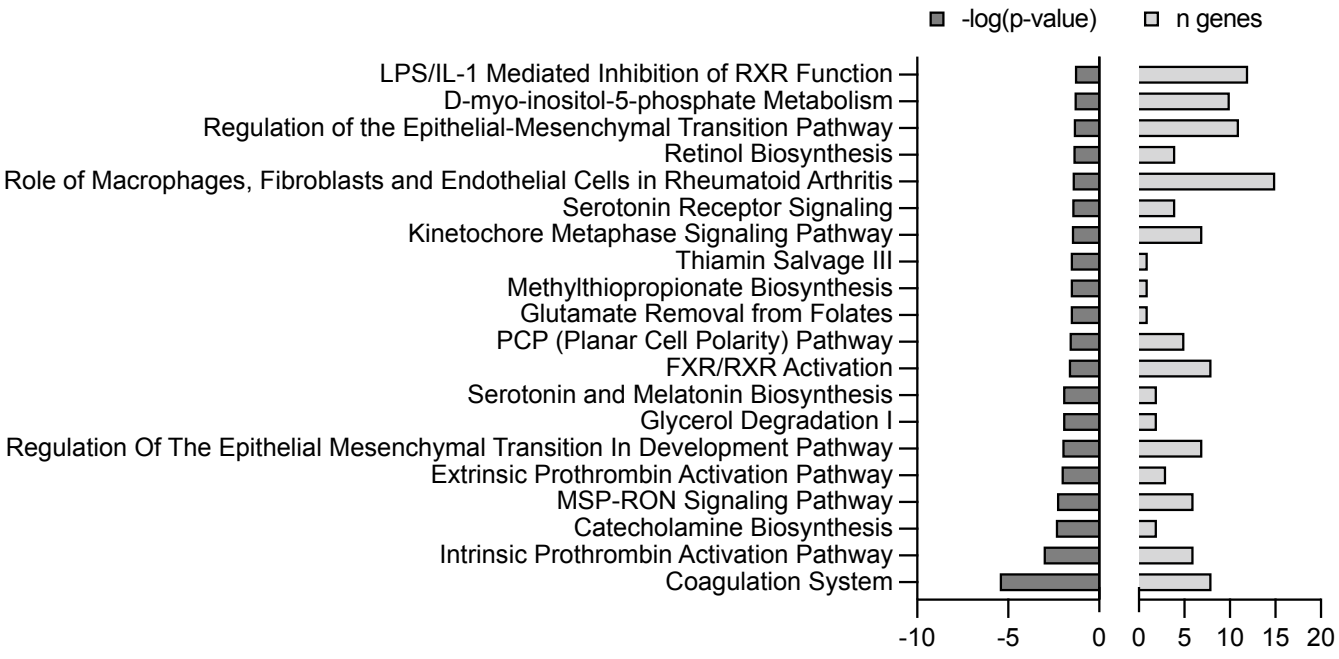

Figure 5-6: Canonical pathways were ranked by $-\log_{10}$(p value) for ESLD phenotype Group 2 (adequate muscle mass, inadequate muscle function) compared to Group 1 (adequate muscle mass and function)

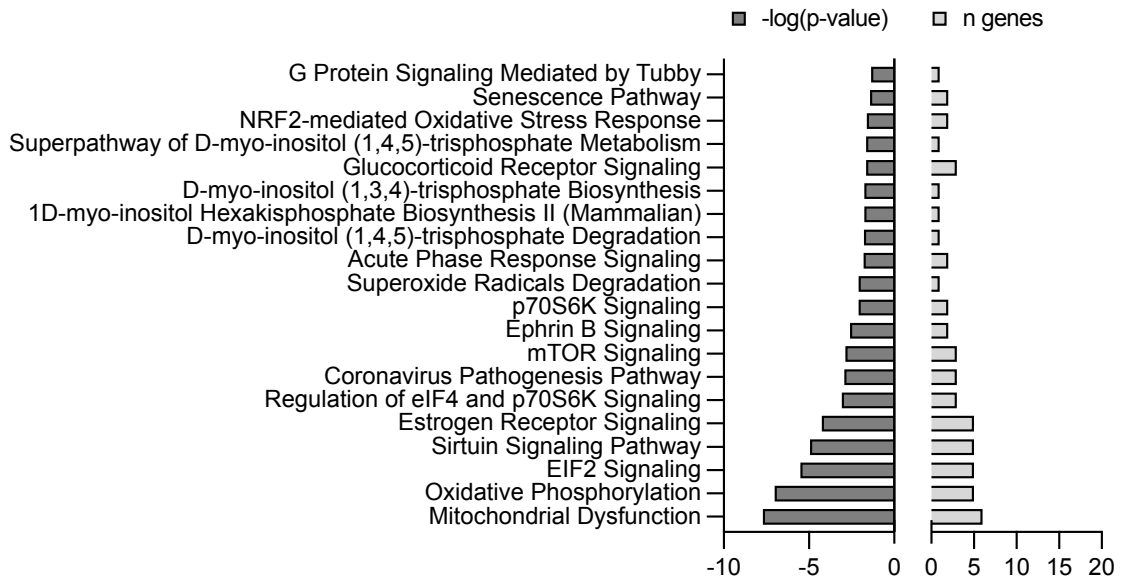

Figure 5-7: Canonical pathways were ranked by $-\log_{10}$(p value) for ESLD phenotype Group 4 (inadequate muscle mass and function) compared to Group 2 (adequate muscle mass and inadequate function)

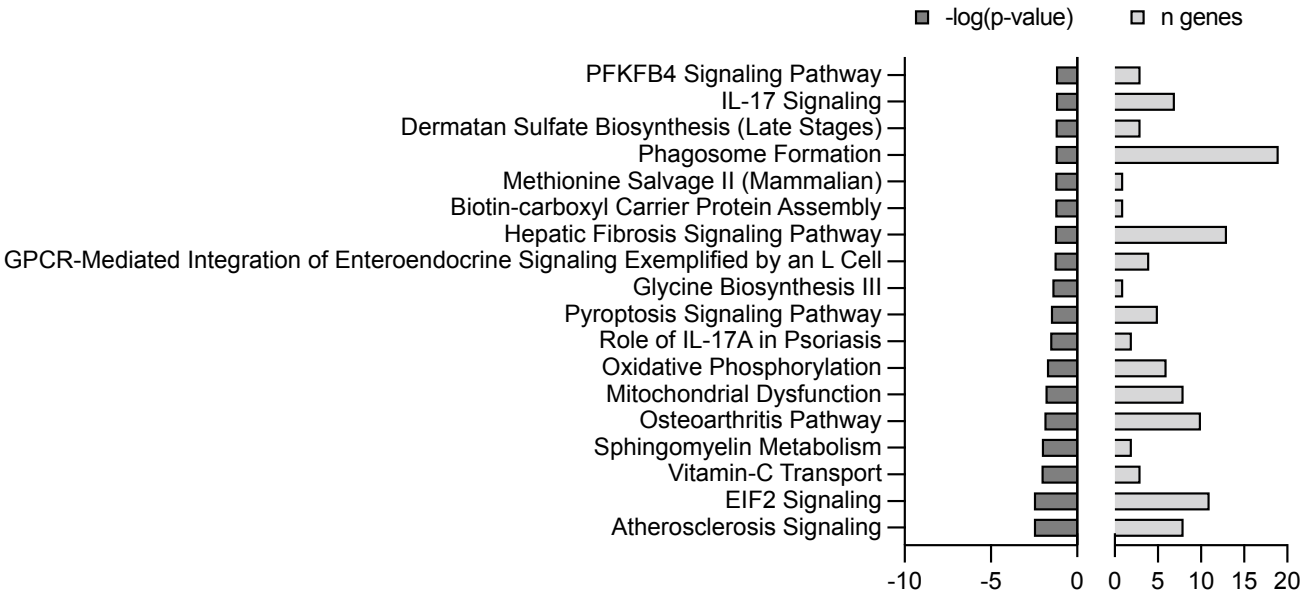

Figure 5-8: Canonical pathways were ranked by $-\log_{10}$(p value) for ESLD phenotype Group 4 (inadequate muscle mass and function) compared to Group 1 (adequate muscle mass and muscle function)

5.4 Discussion

The prospective study is the first, to my knowledge, to consider the concept of muscle wasting phenotypes and muscle to function mismatch, in those with ESLD. Those with inadequate muscle mass and function (Group 4) were older, had a male predominance, and had more advanced liver disease than those with preserved muscle mass and function (Group 1). It is well established that there are age related decline in muscle mass and function (517) in addition to sex related disparities in muscle composition (238, 471, 472). In Chapter 4 it was shown that there were significant reductions in all lower limb measures of muscle mass and knee extensor peak torque of ESLD men compared to healthy controls, demonstrating that there are changes evident within men with ESLD. Furthermore, there is lower testosterone associated in those with ESLD, which can be associated with reduced down regulation of myostatin, an myokine inhibitor of muscle synthesis (283, 289). Further analysis is needed to determine whether sex influences a more severe phenotype of muscle loss. Additionally, those with either a muscle mass to function mismatch (Group 2) or inadequate muscle mass and function (Group 4), had diuretic refractory ascites compared to those with preserved muscle mass and function, who did not. The impact of ascites in ESLD on measures of muscle mass and function in Chapter 4, however these findings again support that ascites appears to be a significant impact on muscle wasting (448).

The analysis between these muscle phenotypes of ESLD does demonstrating significant differences between the measures of muscle mass and muscle function (Table 5.4) which is expected as there are categorised by a difference in muscle mass and function. Those with preserved muscle mass and function (Group 1) had significantly greater muscle mass as measured by quadriceps MRI and ultrasound, greater L3 SMI and higher fat free mass

measured from bio impedance analysis. This suggests quadriceps volume index is a good measure of muscle mass in ESLD as the other commonly used measures of muscle mass such as L3 MSI also showed a significant reduction in those without preserved muscle mass. The measures of muscle function between the ESLD muscle phenotype groups, showed that those with inadequate muscle mass and function (Group 4) or with a muscle mass to function mismatch (Group 2), had significantly lower limb strength, poorer chair stand times, and lower levels of daily activity. The quadriceps possess a greater sensitivity to changes in muscle mass and function; with lower limb strength of a greater importance due to its association with activities of independent living (518-520), which may explain the global reduction in lower limb function and reduced daily activity seen in those with a mass to function mismatch (adequate mass yet inadequate function) and inadequate muscle mass and function.

This study identifies a unique method to define muscle phenotypes patients with ESLD in keeping with the heterogeneity that is observed in routine clinical practice. It was established in Chapter 3 that the lower limb measures of muscle mass demonstrated stronger correlations and difference from healthy controls when compared to conventional truncal measures of L3 SMI. Additionally, it is well established that as one of the largest muscle groups in the body, the quadriceps are highly susceptible to the earliest changes of muscle loss and sarcopenia as demonstrated In the field of primary sarcopenia (436-439), further supporting the use of a lower limb mass measure. Quadricep volume index was selected as it adjusted mass relative to height. Previous research has shown that skeletal muscle mass adjusted by height correlates better with muscle function than mass adjusted by body weight (521) in an older adult cohort. As described in Chapter 3, the strongest measure of lower limb function to quadricep volume index was knee extensor peak torque, therefore this was selected as the

corresponding functional measure. We used our age sex matched healthy controls to define the 25th quartile as that was specific to our cohort, rather than utilising population devised control cut-off, which are non-specific to an ESLD cohort. This is a novel manner for phenotyping these ESLD patients, that requires further validation.

PCA is a commonly used method of reducing dimensionality amongst multiple variables and to aid in identifying trend within the data obtained. The principal component plot findings showed that the variables measured (cytokines, steroids, vitamin D) appeared to demonstrate some clustering of the data. There were clearly demarcated regions for the healthy controls, which supports adequate selection of a healthy control cohort with limited variability. Those with ESLD congregated in a different region of the plot delineated from the healthy controls. This supports a distinct difference in the cytokines, steroid hormones and vitamin D metabolites compared to ESLD.

The loading plot showed that the potential key variables responsible for healthy control clustering included DHEAS, cortisol:cortisone ratio (a measure of 11βHSD1 activity) and PDGF BB, a potent inducer of proinflammatory cytokines (522). DHEAS, a weak androgen that is converted to DHEA, has been shown to influence sensitivity to oxidative stress and insulin sensitivity with a positive effect observed on muscle mass and strength (523, 524). DHEAS, along with other sex steroid hormones decreases with age from the age of 30. Interestingly, studies showed the administration of DHEA showed improved adaption to muscle strength in elderly males (525) with a study by Yanagita et al demonstrating that a cortisol:DHEAS ratio of ≥0.2 is the strongest independent risk factor for sarcopenia in elderly people (526). This suggests that lower levels of DHEAS may be a driver of primary sarcopenia and possibly in our

ESLD cohort. There is conflicting data regarding serum DHEAS levels in liver disease, both elevated and decreased serum DHEAS have been reported in NAFLD, when adjusted for age (524, 527), this warrants further research to explore these findings. Cortisol:cortisone ratio is a marker of net 11βHSD1 activity. 11βHSD1 converts inactive cortisone into active cortisol and is thus a catabolic influence (528). Elevated 11βHSD levels are seen with ageing (529). Studies found that increased cortisol generation within the muscle by 11βHSD may contribute to the loss of muscle strength (529, 530) in ageing cohorts. As 11βHSD1 is commonly found in glucose rich tissues such as the liver, in conjunction with our results, it is plausible that 11βHSD1 has a role in muscle function in those with ESLD, however this needs further exploration. Interestingly 11βHSD1 inhibition has been shown to increase lean mass in obese females (531).

To clinically phenotype the ESLD cohort, we defined an 'adequate' and 'inadequate' threshold for lower limb muscle mass and strength. A clustering of ESLD Group 4 and the healthy controls were seen in the PCA method of the cytokines, steroid hormone and vitamin D metabolites. The analysis did not demonstrate any natural data clusters from the ESLD data that coincided with the clinically phenotypic grouping. There are several potential reasons for this: the ESLD sample size is small and this study may be significantly underpowered to identify influences of these variables on muscle mass and muscle function. During the design of the study, there was little evidence on the prevalence of low muscle mass and function thresholds, to accurate power the study as described in Chapter 2. Additionally, we may need to consider using different variables for our clinically defined phenotypes. As lower limb muscle mass and strength isn't well researched within the field of ESLD, future work could use L3 SMI and HGS (219), which are more frequently used, to define thresholds for adequate

and inadequate mass. Finally, the data may suggest that the early changes seen between the groups occur at a molecular level rather than muscle mass or functional level; in conjunction with the above reasons, further research is required.

In addition to the above, this study, to my knowledge, is the largest number of vastus lateralis muscle biopsies performed in any cohort of ESLD, thus proving both safety and efficacy of the procedure. There was a stringent safety protocol that mitigated against and reduced the risk of adverse events. This experience has been published in further detail (425). Each phenotypic group of ESLD underwent a transcriptomic analysis of the muscle biopsy data to further explore changes in gene expression between the muscle phenotype groups. Differences were seen between the groups themselves from the RNA sequencing data obtained. Those in Group 4, who had inadequate muscle mass and function, demonstrated a clear commonality with mitochondrial dysfunction, oxidative phosphorylation and EIF2 signalling pathway present in both comparisons to Group 1 and Group 2. Sarcopenia is associated mitochondrial dysfunction although the exact mechanisms are not fully understood (232). Ageing impairs mitochondrial function resulting in an increased production of ROS which increases oxidative stress and leads to muscle protein breakdown (532). In age related sarcopenia, there is also a reduction in the quantity of mitochondria, DNA mutations in the mitochondria, reduced maximal oxygen uptake by the mitochondria that results in mitochondrial dysfunction and alterations in mitophagy (533, 534). Furthermore, with ageing, PGC 1 alpha expression, which regulates mitochondrial biogenesis and autophagy to prevent muscle atrophy, is reduced contributing to sarcopenia (534). Therefore, It is interesting that the results showed mitochondrial dysfunction as a stand out signalling pathway in the cohort. In those with ESLD, hyperammonaemia impairs mitochondrial function and promotes oxidative stress by the loss

of alpha ketoglutarate (TCA cycle intermediate) and by increased autophagy, resulting in decreased muscle protein synthesis (251, 281). Similarly to observations described above in age related sarcopenia, reduced number of mitochondria have been observed in patients with Child Pugh B and C cirrhosis (281) suggesting this phenomenon of mitochondrial loss may be an association of both primary and secondary sarcopenia. Furthermore, previous in vivo mouse models of cancer cachexia have shown that mitochondrial dysfunction develops prior to the loss of muscle mass (535) which further supports the changes observed in the analysis reported here.

EIF 2, is an important regulator of protein synthesis and an indirect target of MTORC1 via p70S6K ((536). Again, increased phosphorylation of EIF2 has been shown to impair protein synthesis in hyperammonaemic states (251, 536), supporting this finding in those with a severe sarcopenic phenotype of ESLD. Further analysis of the RNA sequencing suggests strong predominance of pro-inflammatory pathways such as IL 17 signalling, MTOR and its targets (p70S6K) and senescence signalling. Chronic inflammatory conditions such as ESLD, lead to elevated levels of circulating cytokines and increased whole body protein turnover and increased proteolysis (205, 537, 538). In ageing models of sarcopenia, increased muscle autophagy, which can result from mitochondrial dysfunction, can increase levels of pro-inflammatory cytokines through upregulation of Ubiquitin proteasome pathway, further promoting a catabolic state (538). This study thus confirms the potential role of inflammation in muscle wasting phenotypes of ESLD.

The expression of reduced MTOR signalling in this cohort was anticipated. MTOR signalling is a key regulator of muscle protein synthesis and is affected directly by low levels of protein

intake, increased gluconeogenesis in accelerated starvation states, reduced IGF-1, pro inflammatory cytokines, increased myostatin, and hyperammonaemia (232), all of which are seen in those with ESLD. Cellular senescence is also a defining feature of ageing and age-related muscle wasting, with age-related declines in autophagy activation promoting senescence states (539). Senescence itself may initiate the progression of chronic disease with studies showing there is an accumulation of senescent cells promoting chronic liver disease (509, 510). However, there is a paucity of data from research determining potential associations with muscle wasting in chronic liver disease, suggesting further research in the domain is required.

Study limitations

In addition to the limitations highlighted in Chapter 4,regarding the impact of SARS-CoV-2 pandemic, this study has a small sample size for the ESLD cohort and was underpowered for the aims of this study. There were no patients who were categorised in the second group of clinical defined muscle phenotypes (i.e. poor muscle mass with preserved muscle function), hence limiting the analysis between groupings. Additionally this is the first study to define and utilise the concept of dividing those with ESLD into clinically relevant muscle phenotypes using lower limb muscle mass and function. The methodology to do so was based on detailed analyses from Chapter 3 and 4, however the cut offs do require further validation.

End stage liver disease and changes in muscle health are influenced by many factors such as time. The work in thesis is limited to a cross sectional analysis. Further data was collected at 12 and 24 weeks, in addition to the baseline data used here, however due to SARS-CoV-2,

this was outside the constraints of this thesis. It will form the basis for further research assessing changing in muscle phenotypes of ESLD over time.

Finally, it is important to address challenges experienced with performing muscle biopsies within this cohort. The work in this thesis has demonstrated the safety of a muscle biopsy however it was only performed on 23 of the 39 participants in this analysis. The main reason for this was lack of consent. It is recognised that there will always be those who decline a voluntary procedure which may be due to the perception of historical, more invasive methods to obtaining muscle tissue, and the restrictions to activities such as swimming in the immediate short term thereafter. Additionally, there were a small minority of patients who were deemed too high risk due to coagulopathy and concurrent use of anticoagulation (where the risk on an adverse event with cessation of the drug outweighed the need of research muscle biopsy). Further studies utilising this method for muscle biopsies in those with ESLD are required to validate the safety and efficacy of this procedure and to provide grounds for further mechanistic work into the driver behind muscle compromise.

5.5 Conclusion

The premise of clinically phenotyping patients with ESLD by muscle health is a novel approach to cohorting those with inadequate muscle mass and/or muscle function and those with preserved muscle health. It enables us to begin to understand why some patients with ESLD have preserved muscle mass and function and others don't. Even though the cohort size is further limited by sub-grouping it highlights clear differences in age and sex between those with and without preserved muscle health. In addition, conventional methods of disease severity (i.e. UKELD) and aetiology did not appear to contribute to the differences in muscle

health with patients with ESLD. In contrast, there are were clear differences in biological pathways of steroid, pro-inflammation and oxidative stress; with particular dysfunction highlighted in those patients with inadequate muscle mass and function. These results may help to identify key targets in the future in patients with ESLD to not only identify patients in the earlier stages of muscle wasting, but also to develop therapies (pharmaceutical, nutrition) that prevent the loss of both muscle mass and/or function in the future.

6. Chapter Six: General discussion

The concept of muscle health within those with chronic liver disease is an important paradigm which has gained much interest over the past decade. In ESLD, there is a chronic catabolic state that persists driving an imbalance favouring muscle protein breakdown over muscle protein synthesis (205). The only cure for those with decompensated cirrhosis remains liver transplantation, with other management strategies aimed towards stabilising the symptoms and progressions of the disease (162). Therefore, early recognition and management of muscle wasting is an integral aspect of the management of ESLD. One of the most fundamental challenges within this field lies within the definition of sarcopenia and frailty. Most recently, practice guidance by the American Association for the Study of Liver Diseases (AASLD), has formulated operational definitions of sarcopenia and frailty, evolving from theoretical definitions that existed previously. The most validated definition of sarcopenia is the phenotypic presentation of muscle mass loss, with the definition of frailty used to describe elements of muscular contractility, physical performance and function (256).

6.1 Summary of the key findings

One of the primary aims of this thesis was to evaluate the measures of muscle mass and function (strength and performance) in those with ESLD to identify key considerations and methodologies when evaluating those with suspected sarcopenia and frailty. This involved evaluating the correlation of each truncal, upper and lower limb modalities to assess muscle mass, strength and performance within ESLD to compare these to commonly used measures such as L3 SMI and HGS and evaluate their correlation with each other. A second key aim was to compare the differences in these measures of muscle mass, quality, strength and

performance in those with ESLD compared to an age and sex matched healthy control cohort, whilst also investigating the impact of the different aetiologies of ESLD, liver disease severity, age and sex on these measures. Furthermore, the final aim was to define clinical phenotypes of muscle wasting in those with ESLD from the results from the study, and to analyse the variability of the measures of muscle mass, quality and function used with serum cytokine and steroid variables and muscle transcriptomic analysis to identify potential mechanistic drivers of muscle compromise in ESLD with comparisons.

The data from this thesis demonstrates that muscle mass, quality and function should all be considered when evaluating sarcopenia and frailty in ESLD. Figure 6.1 provides an overview of key considerations when evaluating muscle compromise from the results in this thesis and further aspects to explore. The results from Chapter 3 showed that the quadriceps measures of muscle mass from both MRI and US, correlated to the commonly used standard of L3 SMI (219, 222) with MRI quadriceps volume and peak quadricep ACSA possessing the strongest correlation to the other truncal, upper and lower limb mass measures. These data demonstrated the accuracy in these measures being utilised for muscle mass assessment and suggested that the quadriceps measures of muscle mass could be an alternative mode of measurement, avoiding significant radiation from truncal CT imaging. The results also showed that upper limb strength (HGS), the most commonly used and investigated measure of muscle function in those with ESLD (197, 227), correlated well to lower limb strength, supporting the use of knee extensor peak torque as a strength measure. Composite measures of function such as the LFI, also demonstrated significant correlation to measures of HGS and chair stand time used within this score, and other measures of strength (leg extensor peak torque) and SPPB. Again, these results support the use of lower limb measures of mass in ESLD.

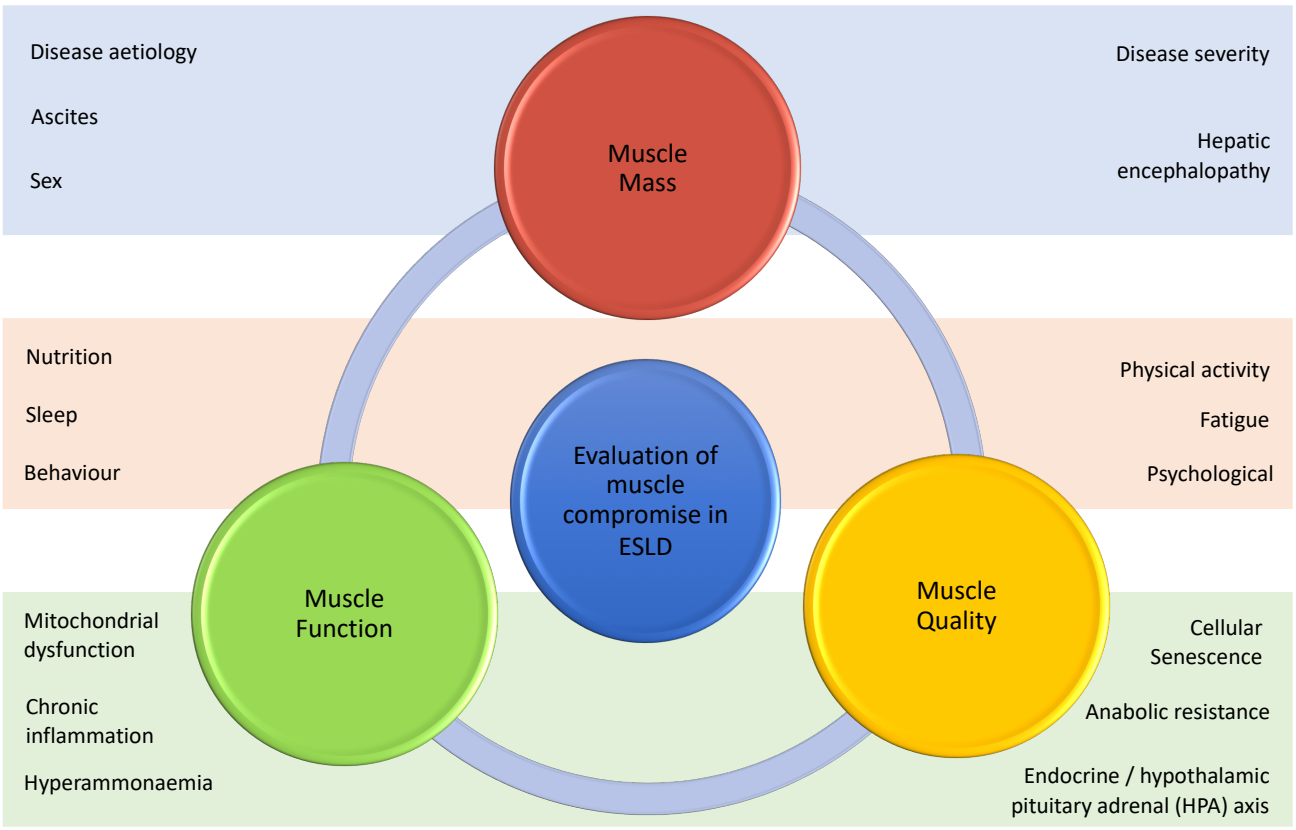

Figure 6-1: Factors affecting the measures of muscle mass, quality and function in ESLD.

The blue box represents fixed variables to consider; the red box represents lifestyle factors influencing muscle mass, quality and function; the green box represents the potential mechanistic drivers of sarcopenia in ESLD.

The quadriceps represent a large muscle group (446) with a significant muscle volume compared to upper limb and truncal mass measures used in ESLD. The lower limb muscle groups undergo earlier changes of muscle compromise in primary sarcopenia (436-439), however in those with ESLD, the lower limb is less susceptible to interference from ascites, and other complications of ESLD, supporting its reliability as a measure of muscle mass. Thus, there should be further consideration of evaluating functionally relevant muscle groups such as the quadriceps to identify those with sarcopenia and/or frailty in ESLD. In this multi-modal assessment of muscle health in ESLD, to my knowledge the first of its kind, the data

demonstrate there is a clear reduction in muscle mass, quality and functional measures in those with ESLD. Differences in muscle mass were only seen in lower limb and not within truncal or upper limb indices, suggesting that compartmentalised muscle changes occur in ESLD. These findings agree with data from primary age-related sarcopenia which has demonstrated preserved upper limb mass in contrast to a greater lower limb muscle mass loss (306, 436). However this remains an area for further evaluation in those with ESLD, with a greater focus on muscle groups such as the quadriceps.

The data in Chapter 4 also reveal there was a global reduction in measures of muscle function in those with ESLD compared to healthy controls (Figure 6.1). Muscle strength was reduced in ESLD in both upper and lower limbs, with lower scores in the composite measures of function, compared to controls, with a greater percentage difference in lower limb strength than upper limb strength, which further supports the notion that increased susceptibility of larger muscle groups such as the quadriceps when evaluating functional muscle loss (436, 439), and the need for further validation of quadriceps muscle mass and functional measures. LFI, which incorporates both lower and upper limb functional measures, showed a greater pre-disposition to frailty in those with ESLD, and provides a greater whole-body assessment of muscle function. Additionally, there was a significant correlation between LFI and total SPPB score. LFI, which was devised for those specifically with liver disease, is exempt from a ceiling effect and may be a more comprehensive test for muscle function. It is an area for future exploration in a UK based cohort of ESLD.

Another key finding from the thesis was a clear increase in myosteatosis in ESLD (Figure 6.1). This may account for the changes in muscle function and performance as the presence of

IMAT can negatively impact force production by disrupting muscle fibre alignments. This can then result in reduced contractility and poorer muscle function (463, 478, 479) and reduced muscle quality has also been shown to be an independent risk factor for mortality (463). Further, IMAT is also associated with metabolic dysregulation including insulin resistance, inflammation and alterations in myokine secretion, which further impair muscle synthesis and drive proteolysis (463, 478, 479). Prior to this thesis IMAT has been under investigated in ESLD, and the results from this study highlight the need for further research into the impact of IMAT on muscle mass and function, and longer outcomes such as mortality. The results also showed that other indicators of muscle quality and contractility vary in ESLD such as specific force (measure of force over mass), pennation angle and PCSA. The preserved specific force showed that both muscle mass and strength is impacted in ESLD, however the low VL pennation angle and PCSA reflects a potential lower amount of contractile tissue and reduced force output. These alterations in muscle quality exist within the lower limb muscle group in those with ESLD though further research is required to validate this finding.

One of the most important findings to highlight is altered physical activity in those with ESLD with an increase in inactive time (a surrogate for sedentary time), compared to controls. Physical activity is a key regular of muscle mass (134) and as such these results highlight that reduced, physical activity is likely an integral driver of muscle wasting in ESLD (276). Inactivity strongly correlates with the development of both primary and secondary sarcopenia (134). The factors behind reduced physical activity and increased inactivity are multifactorial and may include fatigue, reduced energy intake, daytime somnolence, and impaired cognition (489), in addition to low mood and changes in behaviour (Figure 6.1). There are no detailed

studies to date investigating physical activity, excluding step count measures, in those with ESLD, highlighting a major gap in the field for potential research.

Another central consideration in evaluation of muscle health specific to those with ESLD, is the presence of HE or ascites. Within those with and without HE and ESLD, there was a difference in the measures of physical activity compared to controls in this study. There is a paucity of data within this field, most likely due to the ongoing challenge of psychological, cognitive and motor dysfunction that occurs with HE leading to patients with HE being excluded from studies given the variability in their ability to provide consent (458). However, given the undulating nature of HE, those with HE appear to be at an increased risk with the need to explore inclusion of those with HE into studies investigating sarcopenia and frailty. Those with either diuretic controlled or diuretic refractory ascites did show a lower maximal peak knee extensor torque, which may suggest there is a greater negative impact on lower limb muscle function. However only those with diuretic refractory ascites had a lower overall habitual daily activity highlighting the significant impact of ascites on habitual physical activity. Again those with diuretic refractory ascites are an at risk cohort as the increased protein loss from frequency paracentesis, reduced appetite and early satiety from the subsequent compression effects likely to compound loss of muscle mass and function (197). The accumulation of significant ascites is a clear barrier to adequate evaluation of muscle mass and function and requires further elucidation.

Disease aetiology also appears to play an important role in the manifestation of compromised muscle mass and function in this cohort. There is a significant heterogeneity within those with ESLD, with those with ArLD having a more advanced disease phenotype, reduced lower limb

function and strength, a higher IMAT percentage, with lower overall habitual daily activity and a greater inactive time compared to controls. Whilst the mechanism of this remains unclear, the consideration of aetiology should not be overlooked with specific studies investigating the different mechanistic drivers of the differently aetiologies.

This thesis introduced a novel method to define muscle wasting through clinical phenotype defined by adequate or inadequate muscle mass and muscle function. It used a functional approach to defining sarcopenia or muscle wasting, incorporating concepts from EWGSOP and AASLD definitions of sarcopenia and frailty. It is recognised that the sample size is small and the study was not powered for this type of analysis in its original concept. Accepting this limitation, the PCs showed that the variables measured (cytokines, steroids, vitamin D) demonstrated a degree of clustering of the data with clearly demarcated regions for healthy controls and the ESLD cohort in its entirely, although it did not demonstrate natural clustering that coincided with the clinical phenotypic groupings. This suggests that not only are there some molecular changes that predominate, it does suggest that there is some potential in further evaluating this. Stand out variables that may have influenced the principle components included the cortisol: cortisone ratio (a measure of 11βHSD1 enzyme activity) and DHEAS (Figure 6.1). Previous studies found that increased cortisol generation within the muscle by 11βHSD1, driven by induction of 11βHSD1 by inflammatory cytokines, may contribute to the loss of muscle mass and strength (529, 530) in ageing cohorts. With the chronic inflammatory state that exists in ESLD, it is plausible that elevated 11βHSD1 has a role in sarcopenia in those with ESLD. A clinical trial with an 11βHSD1 inhibitor in ESLD would be a good step to address this issue and benefit patients. DHEAS, has been shown to influence

sensitivity to oxidative stress and insulin sensitivity with DHEA supplementation giving a positive effect on muscle mass and strength in older adults (523, 524). Reduced DHEAS levels may therefore play a role in driving muscle compromise and as this steroid has been shown to be safe and is inexpensive, this may represent another novel therapeutic in ESLD.

This is also one of the first studies to explore changes in gene expression between the muscle phenotype groups observed in ESLD. ESLD is a chronic inflammatory condition where elevated pro-inflammatory cytokines have been demonstrated (540). There was a clear predominance of pro-inflammatory pathways, notably IL-17 which would drive infiltration of innate immune cells in to muscle potentially driving localised catabolic pathways. Inflammation is also one of the hallmarks of the biological ageing process (541), driven by processes such as mitochondrial dysfunction and cell senescence, both pathways also revealed in the transcriptomic analysis. It is therefore not unreasonable to propose that the biological ageing of muscle may be accelerated in ESLD, explaining the compromised mass and function. This is one possibility that is now being investigated by the team using approaches such as measuring specific DNA methylation sites known to correlate with biological ageing, termed epigenetic clocks(542). Other pathways such as eIF2, p70S6K signalling and mTOR were observed suggesting muscle ageing and anabolic resistance, akin to that seen in age-related sarcopenia, may also predominate. There is a paucity of transcriptomic data in ESLD highlighting a further area for further exploration within the field.

6.2 Strengths and limitations of the study

The strengths and limitations of the work presented in this thesis are discussed in depth within each chapter. The greatest strength of this study is the multi-modal assessment that was performed producing a novel deep phenotyping of patients with ESLD. Measures of muscle mass, performance and strength were assessed, in addition to blood sampling, vastus lateralis muscle biopsies, actigraphy, food diaries and quality of life evaluation (the latter two aspects have not been included in this thesis). To my knowledge, this is the only study to perform multiple measures of muscle mass and muscle function, of several muscle groups (truncal, upper and lower limb). Additionally, we have evaluated molecular and transcriptomic data in conjunction with these muscle mass and function variables which again is unique and adds a mechanistic perspective that is lacking in those with ESLD and muscle wasting. This translational approach to the study design has allowed for a greater insight in evaluating muscle health in patients with ESLD by assessing commonly used measures of muscle mass and function such as HGS with new approaches such as quadriceps MRI . The exploration into parameters of muscle quality is a key strength of this study. Using multiple variables for quality including IMAT and specific force, has allowed further exploration into the changes described in muscle mass and muscle function, highlighting the importance of muscle quality. This extensive evaluation has provided a unique insight into this cohort into the important considerations involved in muscle health.

The study has shown validated methods for quadriceps measures of muscle mass with MR and US, thus providing a foundation for future research. Furthermore, it is the only study to

date to show the feasibility and safety of vastus lateralis muscle biopsies in patients with ESLD, who often have coagulopathy and thrombocytopenia, deeming the procedure higher risk. Furthermore, the demographics of the patients with ESLD presented in this study represent a real-world sampling of the demographics of UK patients with ESLD, with a similar male predominance and leading aetiology of ArLD, highlighting adequate patient recruitment.

One of the first challenges with this study was due to the definition of sarcopenia. Within ESLD research, sarcopenia is defined as the loss of muscle mass of L3 SMI. Measuring muscle mass and muscle function described in this thesis had never been completed previously in this population against our parameters, therefore calculating an accurate sample size during the design of this study was challenging. Whilst this study is unique, it is underpowered. This is most apparent in the data analysis of the clinically defined phenotypes of ESLD, in Chapter 5. A clustering of controls and overall ESLD patients within the cohort was evident with the cytokine and steroid data, however the lack of natural clustering was not shown with the variables of muscle mass and function, which is likely due to an underpowering of the sample size.

Within the groupings of disease severity via UKELD and age, the categorisation of the patients with ESLD, resulted into uneven groupings which may have affected the significance of the results and caused some challenges when interpreting the data conclusively. A greater sample size and powering as described above is required for the future.

Finally, the greatest challenge was carrying out this study with the emergence of the SARS-CoV-2 pandemic which led to the suspension of all clinical research, including this study. This impacted the study as there could have been further recruitment of the ESLD cohort to account for the patients that withdrew. Further, as discussed in Chapter 4, the healthy control cohort were recruited from the Queen Elizabeth Hospital, Birmingham and the University of Birmingham members of staff, approximately 12 months following recruitment of the ESLD cohort rather than at the same time. Additionally, the work from home when possible mandate, may have reduced physical activity levels and functional results in the healthy cohort.

6.3 Future directions

The work presented in this thesis has described a multi-modal assessment in evaluating muscle health in ESLD. The results have thoroughly evaluated the measures of muscle mass, strength and performance in a cohort of patients with ESLD with the introduction of clinically defined phenotypes and potential mechanistic drivers of muscle loss. The results generated have inevitably led to further questions regarding the evaluation of muscle health in those with ESLD.

In addition to the future considerations described in the previous sections, including the need for combined assessment modalities to incorporate muscle mass, quality and function, from the data collected as part of this study, further exploration into factors such as nutritional intake and quality of life is important. Nutritional intake is an important consideration in the maintenance of muscle mass and function, especially with the phenomenon of accelerated starvation that occurs those with chronic liver disease (244). A 7-14-day food diary was

collected as part of this study, this will be explored in future including relative protein intake, nutritional supplementation, macro and micro nutrient intake. Furthermore, it is recognised that quality of life has an impact on physical activity, physical performance, appetite and sleep, thus incorporation of these data in conjunction with the measures of muscle mass and function in this study may provide additional considerations in the approach to reducing muscle wasting in ESLD.

Further mechanistic studies are also needed, notably to determine if biological ageing is accelerated in ESLD. If correct this gives a very novel avenue for therapeutic intervention as trials are already ongoing using repurposed drugs to low the ageing process(543). This is also true of further histological analysis including muscle fibre typing, satellite cell counts and cross-sectional area which were not carried out and could further confirm the aged phenotype of skeletal muscle in ESLD.

As part of the study, the data from Visit 1 and 2 (weeks 0 and 2) were used. Data were collected at Visit 3 (week 12) and Visit 4 (week 24). As this thesis has provided an analysis of the baseline data from this cohort, a further step would be to evaluate these multi-modal variables over time, including the change in parameters of muscle mass, quality and function over time in those who underwent liver transplantation.

As described above, this study was underpowered, thus it would be important to adequately power a study with a similar multi-modal assessment paradigm, to further elucidate some of the mechanistic drivers that have been highlighted in our clinically phenotyped groups of

ESLD. It is important to further identify and validate this work, as there may be scope for future targeted therapies, such as exercise, pharmaceutical and nutritional supplementation.

6.4 Conclusion

This study has proven that a multi-modal assessment can be performed successfully on a cohort with ESLD. The breadth of data reported suggests that when evaluating changes in muscle composition, muscle mass, quality and function should all be considered in those with ESLD, with support for the use of lower limb measures of mass and function in a functionally relevant muscle group to identify those with sarcopenia and/or frailty. In addition, variables such as disease aetiology, severity, age and sex should be considered. Furthermore, translation of these variables into clinically defined phenotypes and further exploration into the mechanistic drivers of muscle wasting in ESLD requires further work to further the understanding of sarcopenia and frailty.

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8. Appendices

8.1 Publications arising from this thesis

Original articles

- Allen SL, Seabright AP, Quinlan JI, **Dhaliwal A**, et al. The Effect of Ex Vivo Human Serum from Liver Disease Patients on Cellular Protein Synthesis and Growth. Cells. 2022 Mar 24;11(7):1098

- Quinlan JI*, **Dhaliwal A***, et al. Feasibility, efficacy and safety of percutaneous muscle biopsies in patients with chronic liver disease, . Front Physiol. 2022 Feb 15;12:817152. doi: 10.3389/fphys.2021.817152

- **Dhaliwal A,** Williams FR, et al. Evaluation of the mechanisms of sarcopenia in chronic inflammatory disease: protocol for a prospective cohort study. Skelet Muscle. 2021 Dec 11;11(1):27.

Review articles

- **Dhaliwal A**, Armstrong MJ. Sarcopenia in cirrhosis: A practical overview. Clin Med (Lond). 2020 Sep;20(5):489-492.

- Allen SL, Quinlan JI, **Dhaliwal A**, Armstrong MJ, Elsharkawy AM, Greig CA, Lord JM, Lavery GG, Breen L. Sarcopenia in Chronic Liver Disease: Mechanisms and Countermeasures. Am J Physiol Gastrointest Liver Physiol. 2020 Nov 25.

- **Dhaliwal, A**., Williams, F.R., El-sherif, O. *et al.* Sarcopenia in Liver Transplantation: an Update. *Curr Hepatology Rep* 19**,** 128–137 (2020).

- El Sherif O, **Dhaliwal A**, Newsome PN, Armstrong MJ. Sarcopenia in nonalcoholic fatty liver disease: new challenges for clinical practice. *Expert Rev Gastroenterol Hepatol*. 2020;14(3):197-205.

- **Dhaliwal A**, Towey J, Lord JM, Armstrong M, Elsharkawy AM. Nutrition in liver cirrhosis: a case-based overview. *Frontline Gastroenterol*. 2019;11(2):155-161. Published 2019 Sep 10.

8.2 Oral presentations

End-stage liver disease aetiology impacts on the site and severity of muscle weakness in patients awaiting Liver Transplantation: A prospective case-control study - Accepted for on oral presentation at British Liver Transplant Group conference, Leeds, September 2022

8.3 Accepted abstracts

Muscle quality and strength varies between aetiologies in patients with End Stage Liver Disease and sarcopenia – accepted to the Liver Meeting for the American Associated of Liver society annual Liver Meeting, Washington, November 2022

Correlation of trunk, upper and lower limb muscle mass and performance measures in End Stage Liver Disease - accepted to the Liver Meeting for the American Associated of Liver society annual Liver Meeting, Washington, November 2022

The relationship of truncal, upper and lower limb muscle mass, strength and performance in patients with End Stage Liver Disease: A UK prospective study - Accepted for presentation at British Liver Transplant Group conference, Leeds, September 2022