By

ACUTE SARCOPENIA: THE LAST REMAINING ACUTE ORGAN INSUFFICIENCY

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Abstract

Introduction: Acute sarcopenia is defined by incident sarcopenia (muscle insufficiency defined by low muscle strength with low muscle quantity or quality) within six months, normally following a stressor event. It is under-considered in clinical practice, but has potential to lead to reduced quality of life and adverse outcomes from hospitalisation itself.

Methods: I undertook two prospective studies, including (Study 1) hospitalised patients aged 70 years and older, and (Study 2) healthy volunteers aged 18-35. Study 1 involved serial assessments of muscle quantity, quality, and physical function in elective colorectal surgery, emergency abdominal surgery, and general medical (infective diagnosis) patients. Study 2 assessed the variability of muscle quantity and quality measurements performed with Bioelectrical Impedance Analysis (BIA) and ultrasound with position and exercise. In addition, a systematic review was performed to synthesise evidence for interventions to ameliorate negative changes in muscle quantity and function in hospitalised older adults.

Results: Eighty-one participants were recruited (mean age 79, 38.3% female) to Study 1. Serial assessments of muscle quantity, quality, and function were shown to be feasible and acceptable to participants. Variability in trajectories of muscle parameters was demonstrated, with some participants experiencing declines, and others experiencing improvements or recovery across timepoints. Baseline nutritional status and step count were shown to interact in determination of recovery of muscle quantity. Penalised regression models revealed that prescription of steroids was positively associated with sarcopenia at 7 days, and the presence of delirium was negatively associated with change in BATT to 7 days. Forty-four participants (mean age 26, 52% female) were recruited to Study 2. Ultrasound measurements were shown

to increase from the reclined to sitting position and after exercise, demonstrating the importance of ensuring protocol standardisation. Interventions identified within the systematic review included physical activity interventions (27 studies), nutritional interventions (11 studies), testosterone (1 study), GH (2 studies), nandrolone (1 study), erythropoietin (1 study), and Neuromuscular Electrical Stimulation (3 studies). Evidence for effectiveness/efficacy was limited.

Conclusions: Acute sarcopenia research in complex heterogeneous populations of older adults is acceptable and feasible to patients. Targeting interventions to patients most at risk (e.g. those on treatments with steroids) should be considered in future trials. Pragmatic multi-arm trials including physical activity, nutritional, and pharmaceutical agents (e.g. GH) are encouraged.

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The impact of the COVID-19 pandemic on this thesis

The main protocol for this study was originally developed in 2018, and recruitment to the study commenced in May 2019. On March 11th 2020, the World Health Organization declared a global pandemic with outbreak of Coronavirus Disease 2019 (COVID-19) caused by the novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). This was of course entirely unforeseen at the outset of this study, but significantly impacted the conduct of the study, which needed to be adapted in light of this. Due to additional challenges with recruitment at this time, due to need to both protect vulnerable older people from unnecessary contact, and additional clinical service requirements of the researchers during this time, it was not possible to recruit in line with the original target. The impact of the COVID-19 pandemic is discussed as a limitation within each of the results chapters within this thesis.

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Abbreviations

1α,25(OH)2D3	1,25-dihydroxyvitamin D3
25OHD3	25-hydroxyvitamin D3
6MWT	6-minute walking test
ADLs	Activities of Daily Living
Akt	Alpha serine/threonine-protein kinase
ANOVA	Analysis of Variance
ATP	Adenosine triphosphate
BATT	Bilateral Anterior Thigh Thickness
BATT-SCR	Bilateral Anterior Thigh Thickness: Subcutaneous Ratio
BFMI	Body fat mass index
BIA	Bioelectrical Impedance Analysis
BMI	Body Mass Index
CCL2/JE/MCP-1	C-C motif chemokine ligand 2/ Monocyte chemoattractant protein-1
CCL3/MIP-1 alpha	C-C motif chemokine ligand 3/ Macrophage inflammatory protein-1 alpha
CFS	Clinical Frailty Scale
CKD	Chronic Kidney Disease
cm	Centimetre
COPD	Chronic Obstructive Pulmonary Disease
COVID-19	Coronavirus 2019
CRP	C-reactive protein
CT	Computed Tomography

CXCL1/GRO alpha/KC/CINC-1	C-X-C motif chemokine ligand 1/ Ketatinocytes-derived chemokine/ Cytokine-induced neutrophil chemoattractant type-1
CXCL10/IP-10/CRG-2	C-C motif chemokine ligand 10/ Interferon gamma-induced protein 10
DENS Trial	Duration of External Neck Stabilisation Trial
DHEA-s	Dehydroepiandrosterone sulfate
DXA	Duel energy X-ray Absorptiometry
ECW	Extracellular water
eFI	electronic Frailty Index
eGFR	Estimated Glomerular Filtration Rate
eIF-4E	Eukaryotic translation initiation factor 4E
ELISA	Enzyme-linked immunosorbant assay
EMM	Estimated marginal mean
EN	Elastic Net
EWGSOP2	European Working Group on Sarcopenia in Older People 2
FFMI	Fat free mass index
FI	Frailty Index
Flt-2 Ligand/FLT3L	Fms-like typosine kinase 3 ligand
FORCE:SEE	Frailty and Outcomes Record in Clinical Environments: probable Sarcopenia, geriatric Evaluation, and Events study
FoxO	Forkhead box O
g	Grams
GH	Growth Hormone
GLMM	Generalized Linear Mixed Models

GRADE	Grading of Recommendations, Assessment, Development and Evaluations
Hb	Haemoglobin
hsCRP	High sensitivity C-reative protein
ICU-AW	Intensive Care Unit - Acquired Weakness
ICW	Intracellular water
IFN-gamma	Interferon gamma
IGF-1	Insulin-like Growth Factor 1
IL-1 alpha/IL-1F1	Interleukin 1 alpha
IL-1 beta/IL-1F2	Interleukin 1 beta
IL-15	Interleukin 15
IL-4	Inerleukin 4
IL-6	Interleukin 6
IL-7	Interleukin 7
IL-8/CXCL8	Interleukin 8/ C-X-C motif chemokine ligand 8
IQR	Interquartile range
kg	Kilogram
L	Litre
L3	Third lumbar vertebra
m	Metres
m/s	Metres/ second
MAFbx	Muscle Atrophy F-box
METminute	Metabolic equivalent minute
mL	Millilitre
MNA	Mini-Nutritional Assessment

MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
MuRF-1	Muscle Ring Finger-1
N	Number
NFκB	Nuclear Factor Kappa-light-chain-enhancer of activated B cells
ng	Nanograms
NMES	Neuromuscular Electrical Stimulation
pg	Picograms
PHOSP-COVID	Post-hospitalisation COVID-19 Study
PI3K	Phosphatidylinositol-3-kinase
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PROMIS	Patient-reported Outcome Measures Information System
QEHB	Queen Elizabeth Hospital Birmingham
RCT	Randomised Controlled Trial
RF	Rectus femoris
SARCUS	SARCopenia through UltraSound
SC	Subcutaneous
SD	Standard Deviation
SMD	Standardised Mean Difference
SMI	Skeletal Muscle Index
SMM	Skeletal Muscle Mass
SMMJanssen	Skeletal Muscle Mass (Janssen equation)
SMMSergi	Skeletal Muscle Mass (Sergi equation)
SPPB	Short Physical Performance Battery

STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
TBW	Total body water
TNF-α	Tumour Necrosis Factor Alpha
TPA	Total psoas area
TUG	Timed up and go
VEGF	Vascular Endothelial Growth Factor
VI	Vastus intermedius
WCC	White cell count

1 Introduction

1.1 Introduction

1.1.1 Chronic sarcopenia: definitions, mechanisms, and consequences

The definition of sarcopenia has evolved over the last 20 years (Rosenberg, 1997). It is now widely accepted to be defined by both reduced skeletal muscle quantity and reduced muscle function by the European Working Group on Sarcopenia in Older People (Cruz-Jentoft et al., 2010), the International Working Group on Sarcopenia (Fielding et al., 2011), and the Asian Working Group on Sarcopenia (Chen et al., 2014). The European Working Group on Sarcopenia in Older People 2 (EWGSOP2) collaborated to reach a revised consensus definition in 2018 (Cruz-Jentoft et al., 2018). The demonstration of reduced handgrip strength (<16kg females; <27kg males) is defined as probable sarcopenia (Dodds et al., 2014). Additional demonstration of reduced skeletal muscle quantity and/or quality is confirmatory of a diagnosis of sarcopenia. The demonstration of reduced muscle strength, reduced muscle quantity or quality, and reduced physical performance is defined as severe sarcopenia (Figure 1.1-1) (Cruz-Jentoft et al., 2018). Cut-offs are usually derived from two Standard Deviations (SDs) below the mean of a young healthy reference population; 2.5 SDs may be utilised in specific circumstances for a more conservative definition (Cruz-Jentoft et al., 2018). Low muscle quantity or quality without impairment in muscle function has been referred to as presarcopenia; skeletal muscles are still functioning but are on the verge of failure if interventions are not put into place (Cruz-Jentoft et al., 2010).

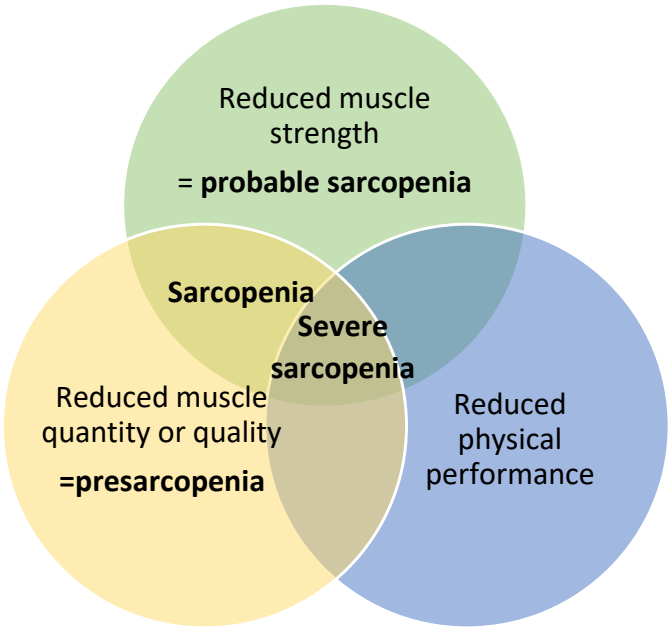

Figure 1.1-1 – Sarcopenia diagnosis as defined by EWGSOP2 (Cruz-Jentoft et al., 2018).

Reduced muscle strength alone is defined as probable sarcopenia; definite sarcopenia is defined by additional demonstration of reduced muscle quantity or quality. Additional case finding of reduced physical performance is defined as severe sarcopenia.

The purpose of skeletal muscle is to generate force. Sarcopenia implies failure of this process leading to insufficient force generation for function. In addition, it is increasingly recognised that the muscle acts as a secretory organ, secreting myokines, cytokines, and other peptides to regulate the immune system (e.g. Interleukin 7 and Interleukin 15 – IL-7, IL-15) (Nelke et al., 2019). Thus, muscle dysfunction can impact systemically; this secretory function may also be affected in sarcopenia. Sarcopenia can be considered as muscle insufficiency, akin to organ insufficiency elsewhere (Cruz-Jentoft, 2016).

Historically, research has focussed upon primary chronic age-related sarcopenia (Cruz-Jentoft et al., 2010), although sarcopenia is now acknowledged to also occur more broadly in the

context of other major organ failure and/or multi-morbidity (Cruz-Jentoft and Sayer, 2019). Chronic age-related sarcopenia is considered to occur secondary to an accumulation of, predominantly inflammatory, insults over time (Dalle et al., 2017). These insults are often subclinical, and relate to a combination of reduced physical activity (Pollock et al., 2018), and age-related disease (Ferrari et al., 2015, Sfyri and Matsakas, 2017). Ageing is associated with cellular senescence; a process whereby cells stop dividing (van Deursen, 2014). Senescence is considered to be protective against cancer by preventing uncontrolled cell division and replication (van Deursen, 2014). However, senescent cells have been shown to be metabolically active and contribute to production of inflammatory mediators (e.g. Tumour Necrosis Factor Alpha – TNF-α, Interleukin-6 – IL-6) (Coppé et al., 2008). Senescence-associated inflammation may drive chronic sarcopenia via activation of Nuclear Factor Kappa-light-chain-enhancer of activated B cells (NFκB) and Forkhead box O (FoxO) in muscle by pro-inflammatory cytokines (De Larichaudy et al., 2012). Activation of NFκB and FoxO drive increased muscle protein degradation via activation of muscle-specific E3 ubiquitin ligases, Muscle Atrophy F-box (MAFbx, atrogin-1) and Muscle Ring Finger-1 (MuRF-1) (Gomes et al., 2001, Bodine et al., 2001). Secretion of Growth Hormone (GH) and Insulin-like Growth Factor-1 (IGF-1) declines with age after approximately 60 years of age; the “somatopause”, which has also been demonstrated in other mammals (Junnila et al., 2013). IGF-1 stimulates muscle protein synthesis via stimulation of the mammalian/mechanistic target of rapamycin (mTOR) via the phosphatidylinositol-3-kinase (PI3K)/ alpha serine/threonine-protein kinase (Akt) pathway, thus reduced IGF-1 secretion with age leads to reduced muscle protein synthesis (Barclay et al., 2019).

The number of motor neurones has been shown to decline with age (Kawamura et al., 1977). However, mechanisms of this are unclear; this may relate to feedback from already dysfunctional muscle, impaired signalling from the central nervous system, local degeneration, or a combination of all of these factors (Gonzalez-Freire et al., 2014). Each motor unit is formed by a motor neurone and the muscle fibres it innervates; multiple units work together to coordinate muscle contraction. In line with motor neurones, motor unit numbers decline with age. This effect occurs independently of physical activity level (Piasecki et al., 2016).

Additionally, prevalence of myosteatosis (intra- and intermuscular fat infiltration) increases with age (Health Aging Body Composition Study, 2009), leading to reduced muscle quality and impaired physical performance (Tuttle et al., 2012). The mechanisms that drive myosteatosis with ageing are unclear. It has been proposed that this may be driven by the differentiation of satellite cells (pluripotent muscle "stem" cells involved in muscle regeneration) into adipocytes, or increased fatty acid transport, uptake, and storage (Miljkovic et al., 2016). Adipose tissue itself secretes pro-inflammatory cytokines (Kern et al., 2001), which further induces muscle protein degradation.

Importantly, the presence of sarcopenia is associated with significantly poorer outcomes, including worse patient-reported quality of life for physical function (Beaudart et al., 2015), increased risk of falls (Bischoff-Ferrari et al., 2015, Beaudart et al., 2017), increased risk of hospitalisations (Bianchi et al., 2015), and increased mortality (Beaudart et al., 2017). The

association of sarcopenia with increased mortality has been shown repeatedly in multiple studies across multiple settings (pooled OR from 11 studies: 3.60, 95% CI 2.96 – 4.37) (Beaudart et al., 2017). Although this association does not necessarily imply causation, it is important to consider that this effect remains even when adjusting for key confounders including age, gender, education, Activities of Daily Living (ADLs) impairment, Body Mass Index (BMI), comorbidities, and systemic inflammation (Landi et al., 2013). Considering the secretory action of skeletal muscle, it is plausible that sarcopenia may directly increase risk of mortality through interaction with other organ systems. Preservation of skeletal muscle may be necessary for prolongation of life, as well as physical function.

1.1.2 Acute sarcopenia: acute organ insufficiency with direct impact on function

The revised EWGSOP2 definition distinguishes acute and chronic sarcopenia (Cruz-Jentoft et al., 2018). Acute sarcopenia is defined as incident sarcopenia within six months, normally following a stressor event (Cruz-Jentoft et al., 2018). As described in section 1.1.1, sarcopenia can be considered as muscle insufficiency (Cruz-Jentoft, 2016); this can be both acute or chronic. In this thesis, I propose that acute sarcopenia should be considered as acute organ insufficiency, akin to acute organ dysfunction elsewhere (e.g. acute kidney injury, delirium) (Welch et al., 2018). Unfortunately, changes in muscle quantity/ quality and function are not routinely measured in clinical practice, and acute sarcopenia has remained poorly characterised to date. The paucity of research that has been conducted has meant that the feasibility of conducting research in complex populations has been unknown, as well as underlying mechanisms, how to stratify risk at patient level, effective intervention strategies,

and associations with longer-term outcomes. Deteriorations in muscle quantity, quality, and function experienced following a stressor event may be partially recoverable, but may increase the risk of chronic sarcopenia over time (Figure 1.1-2) (Welch et al., 2018).

Handgrip strength is recommended for initial assessment for sarcopenia by EWGSOP2, with low muscle quantity or quality being only confirmatory (Cruz-Jentoft et al., 2018). However, this definition was developed with a focus on chronic sarcopenia to enable pragmatic assessment in clinical practice. In the context of acute illness, handgrip strength may be affected by fatigue, impairments in consciousness, and effort (Van Ancum et al., 2017). This may mean that handgrip strength actually increases during the course of illness, representing a recovery from illness fatigue rather than a recovery of muscle function. In addition, acute changes in muscle quantity and quality may occur rapidly, with longer-term impacts on muscle function (Welch et al., 2018). Therefore, measurement of muscle quantity or quality may be especially important in the assessment of acute sarcopenia compared to chronic sarcopenia. Computed Tomography (CT), Magnetic Resonance Imaging (MRI), or Dual-Energy X-ray Absorptiometry (DXA) are recommended for gold standard muscle quantification by EWGSOP2 (Cruz-Jentoft et al., 2018). However, none of these tests can be performed at the bedside, and all have limitations when performed serially (e.g. due to the risk of ionising radiation, physical/psychological burden to patients, cost, and time availability). Bioelectrical impedance analysis (BIA) has been used for the assessment of chronic sarcopenia, but measurements may be affected by fluid balance, potentially limiting its applicability in acute hospital settings (Nakanishi et al., 2019). Ultrasound is recognised as a developing alternative technique for muscle quantity and quality assessment. Ultrasound has benefits in that serial

measurements can be taken with ease in a variety of environments including the outpatient department, inpatient wards, and in the community. It is safe, non-invasive, does not involve ionising radiation, and requires minimal training. However, preliminary research has suggested that muscle quantity and quality may be affected by hypervolaemia in post-operative patients (Welch et al., 2019).

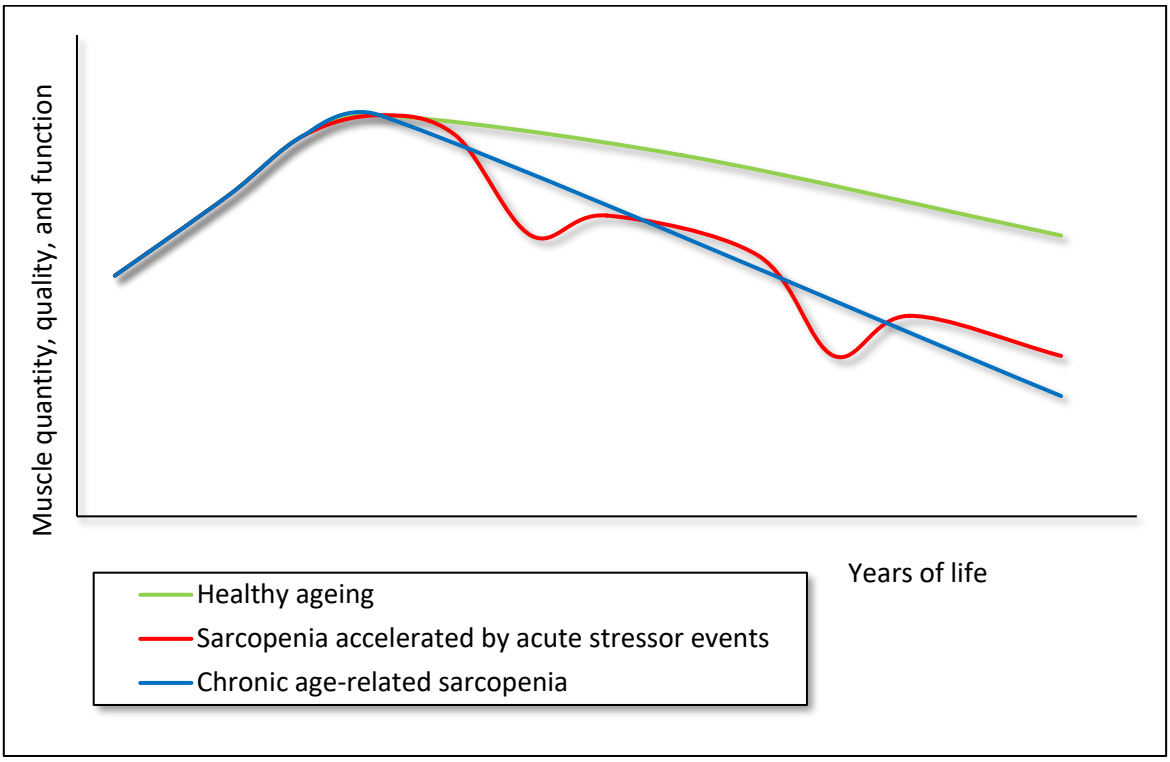

Figure 1.1-2 – Hypothesised trajectories of acute and chronic sarcopenia (adapted from previously published figure (Welch et al., 2018)).

Deteriorations in muscle quantity, quality, and function may be partially recoverable but may be associated with an acceleration in chronic sarcopenia over time.

1.1.3 Mechanisms and drivers of acute sarcopenia

The precise mechanisms involved in the development of acute sarcopenia, and biological and clinical risk factors have remained undetermined (Welch et al., 2018). Determining factors

that are most predictive of risk of acute sarcopenia will enable targeted interventions towards prevention, as well as treatment. Acute sarcopenia has been hypothesised to be caused by a combination of reduced physical activity, increased inflammatory surge, reduced nutritional (protein) intake, and anabolic resistance (blunted muscle protein synthesis with normally recommended protein intake and exercise), with increased vulnerability associated with age, frailty, and impairments to the immune response (Welch et al., 2018).

Frailty is a syndrome of increased likelihood of reduced resolution of homeostasis following a stressor event (Clegg et al., 2013), which is overlapping with, but distinct, from sarcopenia (Dodds and Sayer, 2016). It can be defined based on a physical phenotype (Fried et al., 2001), or based on the accumulation of a number of deficits by a Frailty Index (Rockwood and Mitnitski, 2007). The physical phenotype of frailty is normally considered to be the most closely overlapping with sarcopenia (Dodds and Sayer, 2016), as it is defined by the presence of three out of five characteristics of reduced muscle strength, reduced gait speed, weight loss, self-reported exhaustion, and low energy expenditure (Fried et al., 2001). The prevalence of frailty increases with age, but it is not an inevitable aspect of ageing. Ageing and frailty are both associated with impairments in the immune response, termed “immunesenescence” (Wilson, 2018). Such impairments in the immune response could lead to impairments in muscle metabolism. Following damage, immune cells are recruited into muscles in order to initiate pathogen clearance and tissue repair (Pillon et al., 2013). Frailty has been shown to be associated with impaired migration of neutrophils and other immune cells, meaning this process of tissue repair within muscles with illness may be impaired, potentially increasing the risk of acute sarcopenia in this vulnerable population (Wilson et al., 2020).

Hospitalisation is frequently associated with reduced physical activity and periods of bedrest. Studies involving healthy volunteers have demonstrated that bedrest is associated with declines in muscle quantity, strength, and aerobic performance, and that this effect is exacerbated by age (Kortebein et al., 2007, Tanner et al., 2015). Bedrest has been shown to lead to reduced muscle protein synthesis via altered expression of MAFbx and MuRF-1 (not timed with feeding) (Jones et al., 2004), increased insulin resistance (Hamburg et al., 2007), and reduced oxidation of saturated dietary fat (Bergouignan et al., 2006) in healthy younger adults. However, it is not known how skeletal muscle "senses" bedrest to precipitate these effects (Crossland et al., 2019).

Acute illness (e.g. acute bacterial infection, and recently the Coronavirus Disease 2019 [COVID-19] pandemic (Welch et al., 2021)) and major surgery are associated with endocrinological stress responses (e.g. increased cortisol, decreased dehydroepiandrosterone sulfate (DHEA-s)) (Butcher et al., 2005). Medically-induced hypercortisolaemia (hydrocortisone injection) has been shown to exacerbate loss of muscle quantity during bedrest in healthy young adults (Paddon-Jones et al., 2006) and synthetic glucocorticoid (dexamethasone) has been shown to upregulate MuRF-1 and MAFbx 10-fold in rodent models (Bodine et al., 2001). Preliminary research suggested that baseline DHEA-s serum concentration levels may correlate with declines in physical performance experienced during hospitalisation (Welch et al., 2019).

Acute illness and surgery are also associated with a heightened systemic inflammatory response (Smeets et al., 2018). Inflammation associated with acute illness reduces muscle protein synthesis. TNF-α has been shown to decrease messenger Ribonucleic Acid (mRNA) translational efficiency through alterations in Eukaryotic translation initiation factor 4E (eIF-4E) availability (Lang et al., 2002). This leads to a state of anabolic resistance whereby higher protein doses are needed to stimulate an adequate response. This effect is also compounded by the effects of bedrest, regardless of inflammation. Fourteen days of reduced physical activity in healthy older adults has been shown to be associated with reduced postprandial rates of muscle protein synthesis (Breen et al., 2013).

Unfortunately, many older adults have reduced baseline nutritional status, with studies showing that a third of older adults are at risk of malnutrition on admission to hospital (Pierik et al., 2017, van Vliet et al., 2020). Additionally, nutritional intake frequently declines during acute illness, particularly in older adults, due to age- and illness-related anorexia (Landi et al., 2016), physical limitations (Simmons and Schnelle, 2004), and swallowing difficulties (Jardine et al., 2018). This may lead to ineffective protein intake for muscle protein synthesis, which will be compounded by higher protein requirements with acute illness-related inflammation, and reduced physical activity during hospitalisation.

Vitamin D has been implicated in the regulation of muscle mass and function. Vitamin D receptor mRNA has been demonstrated in skeletal muscle and this negatively correlates with inactive 25-hydroxyvitamin D3 (25OHD3) serum concentration levels in healthy adults aged

20 to 74 years (Hassan-Smith et al., 2017). Active serum 1,25-dihydroxyvitamin D3 (1α,25(OH)2D3) concentration levels correlated with lower limb muscle strength in this study (Hassan-Smith et al., 2017). Vitamin D inhibits FoxO-mediated transcriptional activity to prevent muscle protein degradation and, thus, induces muscle atrophy (Hirose et al., 2018). Vitamin D deficiency and insufficiency are prevalent amongst older adults. The cause for this is often multifactorial and relates to a combination of reduced ultraviolet light (sun) exposure, reduced ability to effectively synthesise Vitamin D with ultraviolet light, reduced nutritional intake, and kidney impairment impairing Vitamin D metabolism. Additionally, plasma 25OHD3 concentration levels have been shown to decline following elective surgery, with this change correlating with corresponding increases in C-Reactive Protein (CRP), a potent marker of inflammation (Reid et al., 2011). Therefore, vitamin D deficiency or insufficiency may be especially common in older adults following surgery or with inflammation arising from acute illness, contributing towards the development of acute sarcopenia.

Older adults, particularly those living with frailty, are also at increased risk of development of delirium during acute illness and hospitalisation (Persico et al., 2018). Delirium is an acute severe neuropsychiatric condition, which is increasingly recognised to be associated with systemic inflammation (Vasunilashorn et al., 2015). These inflammatory processes may directly communicate with skeletal muscle to increase the risk of acute sarcopenia. This is combined with the cognitive effects of delirium, which may directly impact upon the initiation of motor control.

Acute illness may also lead to a state of “induced frailty”, which is associated with systemic inflammation and catabolism (Hawkins et al., 2018). Induced frailty is an advancing research area, which, similar to acute sarcopenia, has not been fully characterised to date. Frailty is commonly assessed by considering a patient’s physical and cognitive function two weeks prior to hospitalisation, to allow for reversible declines in function related to acute illness (Rockwood and Theou, 2020). However, it is widely accepted that frailty is a dynamic process (De Lepeleire et al., 2009). In some patients, the process of hospitalisation in itself may lead to increasing severity of frailty. Whilst this state should be managed and considered differently to frailty that develops over months or years, it could be postulated that this may be associated with further impairments to the immune system, leading to increased risk of adverse outcomes (Hawkins et al., 2018). In particular, immune dysfunction may further impact upon tissue repair, and persistent inflammation will reduce muscle protein synthesis and increase muscle protein breakdown, accelerating the development of acute sarcopenia.

Although acute declines in muscle quantity, quality, or function may be experienced during acute illness at any age, it is this interplay between ageing, immunesenescence, inflammation, and acute illness factors that makes older adults most vulnerable. Figure 1.1-3 demonstrates how these mechanisms and drivers interact.

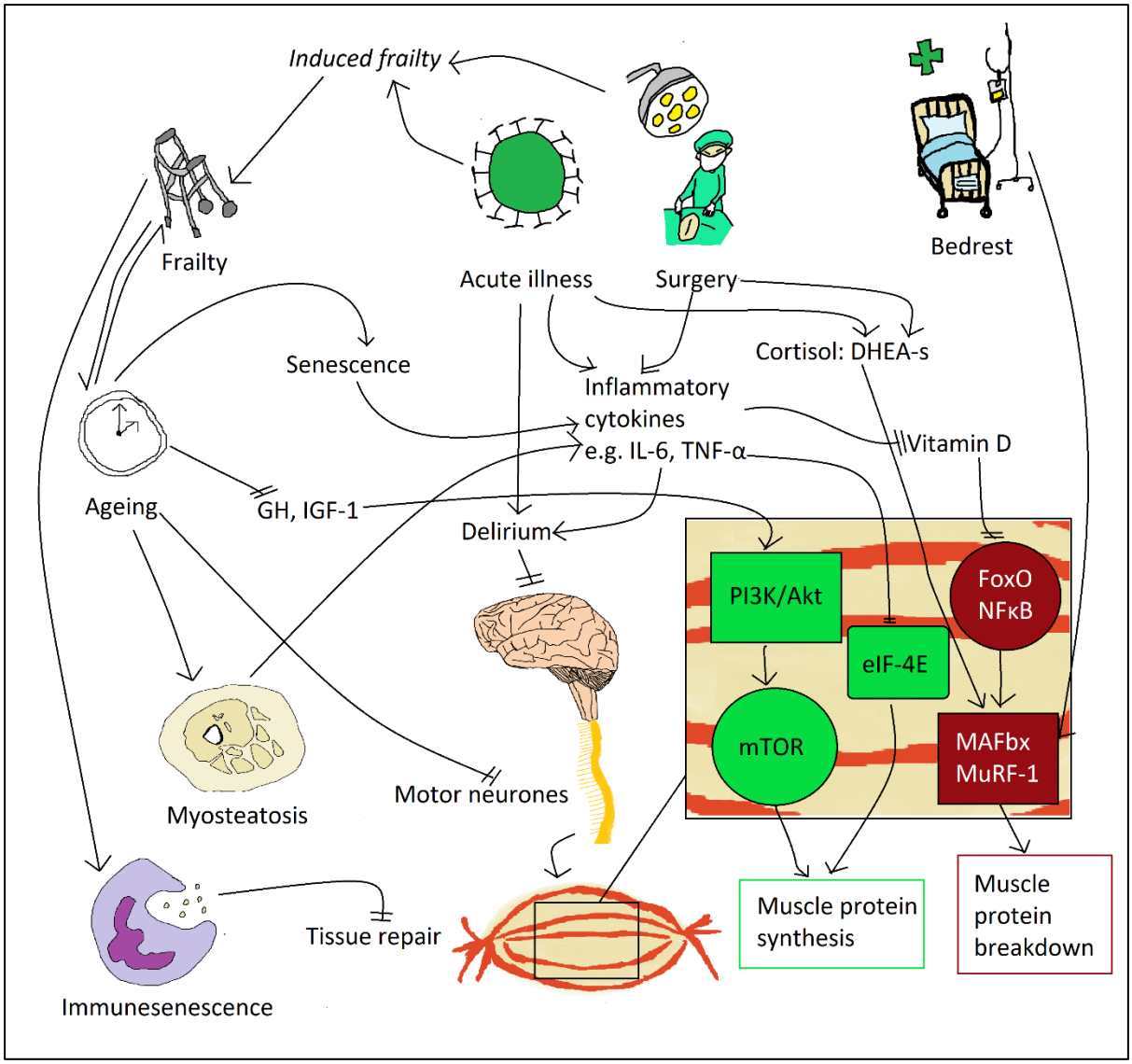

Figure 1.1-3 – Mechanisms and drivers of acute sarcopenia in hospitalised older adults.
Acute sarcopenia is considered to be precipitated by a combination of heightened inflammation and bedrest, on a background of age-associated vulnerability. This leads to an imbalance in muscle protein synthesis and breakdown. Pointed arrows show pathways of promotion; blunted arrows show pathways of inhibition.

Conversely, there is increasing evidence that older adults with chronic sarcopenia and severely reduced muscle quantity experience minimal further declines in muscle quantity during periods of immobility (Lunt et al., 2021). Bedrest studies have involved participants who were far younger and fitter than the typical population of older adults admitted to

hospital within developed countries; these studies did not involve participants with frailty or chronic sarcopenia (Kortebein et al., 2007, Tanner et al., 2015). This “end-stage” of muscle organ dysfunction may lead to blunted responsiveness from communication between the muscle and immune system. In the presence of pronounced chronic sarcopenia, the muscle may no longer respond and react to systemic inflammation. This is important as this group of individuals may require a different focus of treatment to prevent further loss of function. Increased understanding will ensure that clinicians can appropriately prognosticate for their patients, and that the information patients are given is correct and of relevance to them.

1.1.4 Consequences of acute sarcopenia

Whilst acute sarcopenia remains poorly categorised, potential consequences can be postulated on the basis of the clinical outcomes of chronic sarcopenia. Acute sarcopenia can be expected to increase risk of falls, as has been repeatedly demonstrated with chronic sarcopenia (Beaudart et al., 2017, Bischoff-Ferrari et al., 2015). Falls can be potentially devastating in the unfamiliar environment of hospitalisation. Infection control policies in hospital necessitate hard surfacing to flooring, leading to significant risk of injury in patients who do fall (Simpson et al., 2004). The effect of chronic sarcopenia upon length of hospital stay is unclear with some studies showing increased length of stay (Sousa et al., 2016), and others not showing a clear association (Sánchez-Rodríguez et al., 2015). However, regardless of the effects of sarcopenia upon trajectories of illness, patients who experience significant declines in physical function during hospitalisation will normally require prolonged inpatient

rehabilitation (Freburger et al., 2020). Increased length of stay is associated with particular risk to older adults with increased vulnerability to nosocomial infections (Avci et al., 2012), potentially leading to a "vicious cycle" in the development of acute sarcopenia. It should also be considered that sarcopenia does not only affect limb muscles, and the concept of sarcopenic dysphagia is increasingly recognised, i.e., weakness of swallowing muscles secondary to underlying age- and illness-related processes (Fujishima et al., 2019). Sarcopenic dysphagia may lead to reduced nutritional intake, exacerbating the effects of acute sarcopenia, and increased risk of aspiration and further infections (Fujishima et al., 2019).

Considering the potential effects of acute sarcopenia upon increased risk of complications (e.g. infections, injuries from falls), it is conceivable that acute sarcopenia may be associated with increased mortality. This is in addition to the known independent associations of sarcopenia with increased mortality in general (Beaudart et al., 2017, Landi et al., 2013). The reason for this independent association is unclear. However, if the secretory action of skeletal muscles is affected in sarcopenia, it can be postulated that this may be associated with worse outcomes through interactions with other organ systems. In survivors, acute sarcopenia may be associated with increased care needs on discharge, necessitating increased social care costs. In participants who do not experience recovery of function, and who progress to development of chronic sarcopenia, this will be associated with reduced quality of life (Beaudart et al., 2015). Figure 1.1-4 pictorially demonstrates the potential consequences of acute sarcopenia.

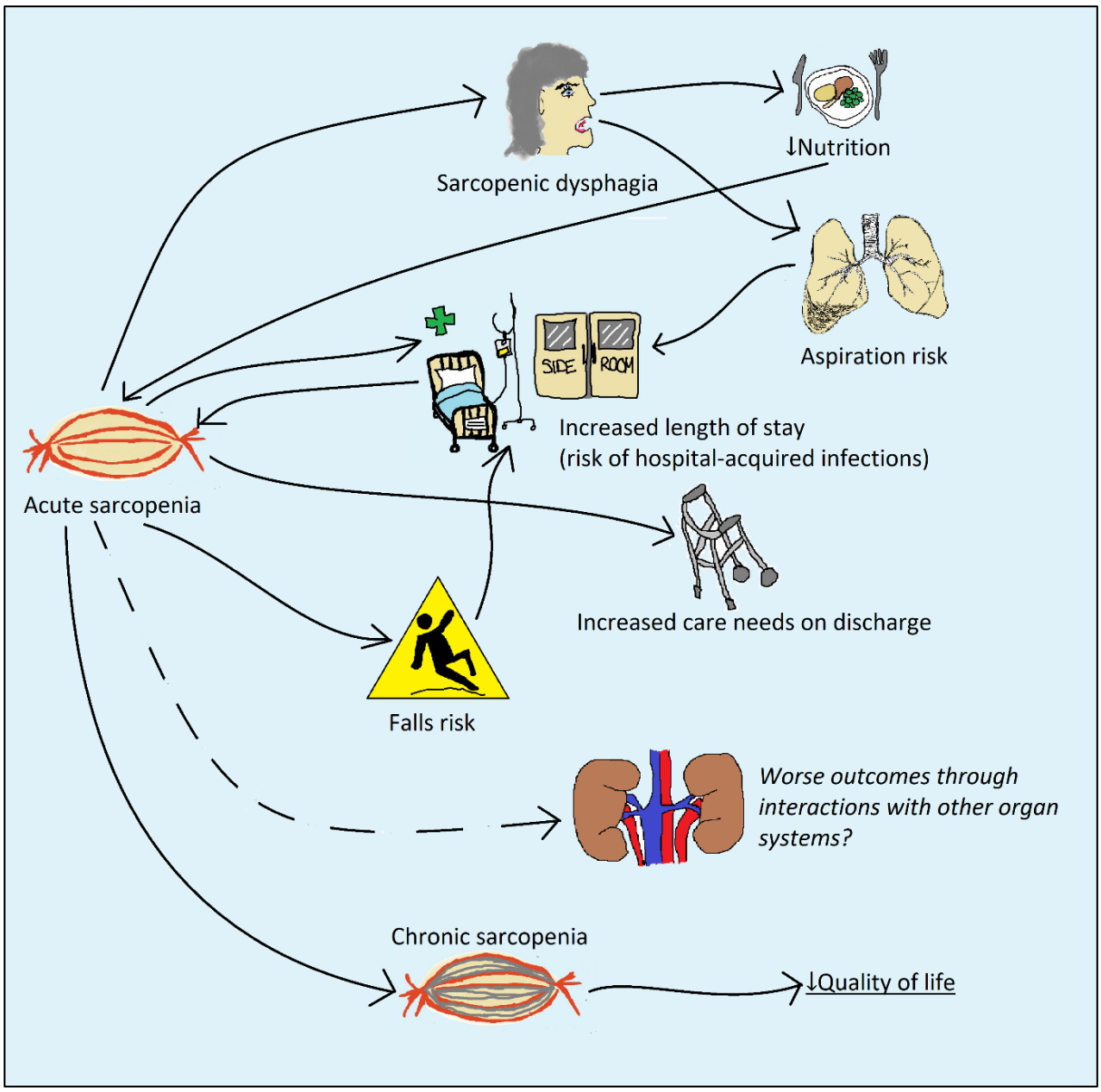

Figure 1.1-4 – Consequences of acute sarcopenia.

Whilst acute sarcopenia has not been fully characterised, potential consequences can be postulated. The risk of further illness and injuries from falls may lead to a "vicious cycle" of acute sarcopenia development in some patients. Unbroken pointed lines show pathways of promotion considering current best evidence. The broken pointed line shows a theoretical association, for which further research is needed to evaluate this pathway.

1.1.5 Proposed interventions for acute sarcopenia

As acute sarcopenia has been poorly characterised to date, most effective prevention and intervention strategies are unknown (Welch et al., 2018). However, it is conceivable that effective interventions will target some or all of the potential mechanisms described. This is likely to include a combination of physical activity (e.g. resistance exercise to combat negative effects of bedrest), nutritional (e.g. high protein supplementation in view of anabolic resistance), and pharmaceutical interventions (e.g. vitamin D, DHEA-s, immune-modulatory agents). Alternative interventional strategies to target specific muscle groups could involve direct simulation of the muscles using massage (Lawrence et al., 2020), vibration therapy (Wu et al., 2020), low level laser therapy (Toma et al., 2016), or Neuromuscular Electrical Stimulation (NMES); the latter uses low-frequency, low-amplitude electrical current to activate motor neurones resulting in muscle contraction (Welch et al., 2018). Characterisation of changes in muscle quantity and function in hospitalised older adults will enable risk stratification and understanding of mechanisms, towards targeted interventions.

1.1.6 Research questions

The projects described in this thesis will address the following questions:

1. What is the feasibility of conducting acute sarcopenia research in complex heterogenous groups of hospitalised older patients?

2. What is the acceptability of performing tests of muscle quantity, quality, strength, and physical performance during and post-hospitalisation for heterogenous groups of older adults?

3. How does hospitalisation and acute illness and/or surgery impact upon muscle quantity, quality, strength, and physical performance in heterogenous groups of older adults at one week and three months?

4. What is the incidence of acute sarcopenia in hospitalised older adults, and how do dynamic changes relate to dynamic changes in frailty status?

5. What biological and clinical factors are predictive of changes in muscle quantity, quality, strength, physical performance, and patient-reported outcomes in heterogeneous groups of hospitalised older adults at one week and three months?

6. What is the current evidence for interventions to ameliorate negative changes in muscle quantity, strength, and physical performance in hospitalised older adults?

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2 Methods of muscle assessment and sarcopenia evaluation

Chapter 2.1 - Protocol for understanding acute sarcopenia: a cohort study to characterise changes in muscle quantity and physical function in older adults following hospitalisation

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2.1 Protocol for understanding acute sarcopenia: a cohort study to characterise changes in muscle quantity and physical function in older adults following hospitalisation

2.1.1 Abstract

Background: Older adults are vulnerable to the effects of acute sarcopenia (acute muscle insufficiency) following hospitalisation. However, this condition remains poorly characterised to date. It is hypothesised that acute sarcopenia arises due to a combination of bed rest and inflammatory surge. This study aims to characterise changes in muscle quantity and function, determining which factors (clinical and biological) are most predictive, and how these relate to change in physical function at 13 weeks.

Methods: This study will include three groups of patients aged 70 years and older; patients undergoing elective colorectal surgery, patients admitted for emergency abdominal surgery, and patients admitted under general medicine with acute bacterial infections. Changes in muscle quantity (Bilateral Anterior Thigh Thickness with ultrasound and bioelectrical impedance analysis) and muscle function (muscle strength, physical performance) within one week of hospitalisation or surgery will be characterised, with follow-up of patients at 13 weeks. Physical function will be measured using the Patient Reported Outcome Measures Information System, and the Short Physical Performance Battery (or gait speed alone within one week of surgery).

Discussion: This study will fully characterise changes in muscle quantity and function in hospitalised older adults and enable risk stratification towards targeted interventions in

clinical practice. The results of this study will inform further research involving interventions to ameliorate changes.

Trial registration: ClinicalTrials.gov Identifier: NCT03858192; Prospectively registered 28th February 2019

2.1.2 Background

Acute sarcopenia is an emerging condition of acute muscle insufficiency; older adults are considered particularly vulnerable to its effects following hospitalisation (Welch et al., 2018). The European Working Group on Sarcopenia in Older People 2 (EWGSOP2) defines sarcopenia as reduced muscle strength with reduced muscle quantity or quality; cut-off values to meet criteria are usually set 2.0 – 2.5 standard deviations below the mean of a young adult healthy reference population. Additional demonstration of low physical performance is defined as severe sarcopenia. The revised definition (EWGSOP2) includes a distinction between acute and chronic sarcopenia; acute sarcopenia is defined as incident sarcopenia within six months, normally following a stressor event (Cruz-Jentoft et al., 2018). However, acute sarcopenia has been poorly characterised to date (Welch et al., 2018).

The biological mechanisms, clinical risk factors, longer term outcomes, and most effective management strategies of acute sarcopenia are currently unknown. Acute sarcopenia is considered to be caused by a combination of heightened inflammation and muscle disuse during bedrest. Studies involving healthy volunteers have demonstrated that bedrest is

associated with declines in muscle quantity, strength, and aerobic performance, and that this effect is exacerbated by age (Kortebein et al., 2007, Tanner et al., 2015). Acute illness (e.g. acute bacterial infection) and major surgery are associated with systemic inflammatory response (Smeets et al., 2018) and endocrinological stress response (e.g. increased cortisol, decreased dehydroepiandrosterone sulfate (DHEA-s)) (Butcher et al., 2005). Pro-inflammatory cytokines activate pathways leading to increased muscle protein degradation (De Larichaudy et al., 2012) and hypercortisolaemia has been shown to exacerbate loss of muscle quantity during bedrest (Paddon-Jones et al., 2006). It has been postulated that acute sarcopenia may be partially recoverable, but may increase the risk of chronic sarcopenia over time (Welch et al., 2018). It is proposed to be related to a combination of acute inflammatory surge and bedrest during hospitalisation (Welch et al., 2018). Characterising acute sarcopenia will enable greater understanding of the significance of changes in clinical practice, and allow risk stratification towards targeted interventions.

EWGSOP traditionally recommended Computed Tomography (CT), Magnetic Resonance Imaging, or Dual-Energy X-Ray Absorptiometry to measure muscle quantity (Cruz-Jentoft et al., 2010). However, these tests cannot be used at the bedside and have limitations when used serially (Biswas, 2009). Ultrasound measurement of Bilateral Anterior Thigh Thickness (BATT) has excellent inter-rater and intra-rater variability (Wilson et al., 2019). EWGSOP2 supports use of ultrasound for clinical assessment of sarcopenia (Cruz-Jentoft et al., 2018) and a consensus protocol has been proposed (Perkisas et al., 2018). Bioelectrical Impedance Analysis (BIA) is an alternative non-invasive tool that provides estimates of lean mass. Muscle quantity measured by BIA has been shown to correlate with BATT (Wilson, 2018), however,

BIA is more greatly affected by fluid balance (Ticinesi et al., 2017, Nakanishi et al., 2019). BIA is also not currently recommended for use on people with implantable cardiac devices, although research suggests this is likely to be safe (Chabin et al., 2019).

Colorectal surgery is commonly performed on older adults (The Royal College of Surgeons of England, 2012). It is not typically associated with cachexia, when performed for localised colorectal cancer (Fox et al., 2009); metastatic cancer is known to be associated with increased risk of cachexia compared to localised cancer (Shiono et al., 2016). Colorectal surgery patients do not typically present with disease-associated pre-operative functional decline (Smith et al., 2006), as compared to orthopaedic or vascular surgery, where impairments in function are presenting symptoms of the illnesses themselves (Regensteiner et al., 1993, Kapstad et al., 2007). This offers the opportunity for pre-insult measurements to be taken prior to hospitalisation. Previous studies have demonstrated acute declines in handgrip strength and muscle quantity using BIA in older adults admitted electively for colorectal surgery (Van Ancum et al., 2017a). Acute reductions in BATT and usual gait speed were also demonstrated in our pilot study, which was used to refine this protocol (Welch et al., 2019). Interestingly, an apparent increase in BATT was demonstrated immediately postoperatively (Welch et al., 2019); this may be related to fluid balance but warrants further investigation (Fischer et al., 2016). However, emergency admitted patients may be at the greatest risk of declines in muscle quantity and function due to increased inflammation. Within the UK, hospitalised older adults are most commonly admitted to general medicine wards (NHS Digital, 2019). Studies involving medical and orthopaedic patients have shown variable changes in muscle quantity and function in hospitalised older adults (Van Ancum et

al., 2017a, Van Ancum et al., 2017b, Martone et al., 2017), and changes have not been evaluated in patients admitted for emergency abdominal surgery.

2.1.3 Methods

2.1.3.1 Aim

To clinically and biologically characterise acute sarcopenia in older hospital populations, assessing for within group differences in elective colorectal surgery, emergency surgery, and general medicine patients. This will enable determination of mechanisms and identification of potential intervention strategies.

2.1.3.2 Design and setting

This is a single site cohort study at the Queen Elizabeth Hospital Birmingham (QEHB), involving 56 elective colorectal, 56 emergency abdominal surgery, and 56 medical patients. QEHB is a large tertiary hospital, with a firmly embedded research infrastructure. In the elective cohort, measurements will be performed in preoperative assessment clinic, within 48 hours of surgery, at 7 days postoperatively (+/-2 days), and at 13 weeks postoperatively (+/-1 week). In the emergency surgery cohort, we aim to recruit participants preoperatively where possible. Where this is not possible, we will recruit participants within 48 hours of emergency surgery; further assessments will be performed at 7 days postoperatively (+/-2 days), and at 13 weeks postoperatively (+/-1 week). Medical patients will be recruited within 48 hours of

admission with further assessment at 7 days post-admission (+/-2 days), and at 13 weeks post-admission (+/- 1 week). The timeframe of 13 weeks has been chosen pragmatically as a timeframe that was considered important to our patient and public involvement panel that could be feasibly conducted without high drop-out rates. The full study schema is shown in Figure 2.1-1.

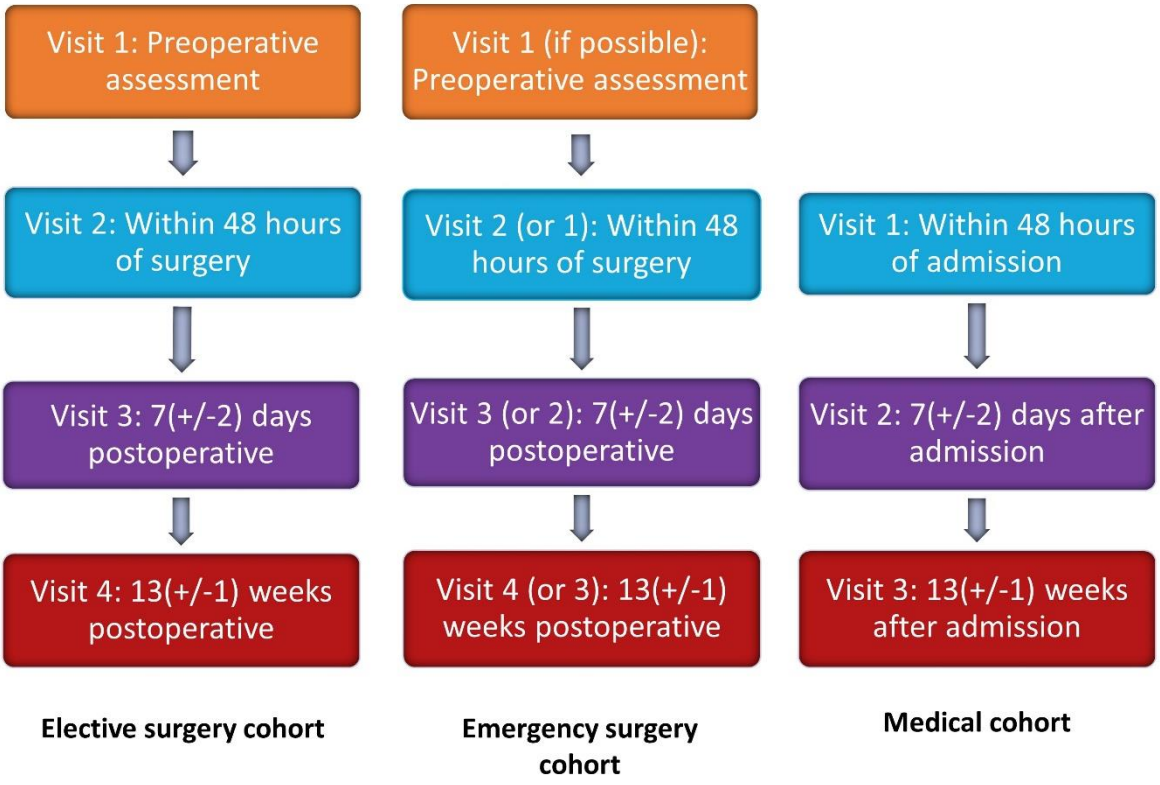

Figure 2.1-1 - Study schema for recruitment and follow-up of each included cohort

2.1.3.3 Characteristics of participants

The elective cohort will include patients expected to undergo major colorectal surgery, the emergency surgery cohort will include emergency admitted patients who have undergone or

are planned to undergo emergency abdominal surgery, and the medical cohort will include emergency admitted patients with (or suspected) acute bacterial infections. Participants aged 70 years or older at time of recruitment will be included in all cohorts. Participants who are unable to provide written informed consent at time of recruitment will not be included in the elective cohort, although specific consent will be obtained for participants to remain in the study if they lose capacity, including details of any named consultee. In the emergency surgery and medical cohorts, personal or professional consultee declaration will be obtained if the participant is unable to provide written informed consent. Participants who are unable to understand verbal English, who were unable to mobilise prior to admission to hospital, or who have a life expectancy of less than 30 days will be excluded from all cohorts.

2.1.3.4 Processes and interventions

Table 2.1-1 shows the complete schedule of assessments that will be performed during this study. We describe the procedures that will be performed at each visit in further detail below.

Table 2.1-1 – Schedule of study procedures

This chart shows all possible visits for each cohort and assessments that would be expected to take place for each participant in each cohort. Visits marked with * may not take place for all participants.

	Elective cohort				Emergency cohort				Medical cohort		
Visit	A	B	C	D	A*	B	C	D	B	C	D
Demographics, observations, medications, medical history,	✓	✓	✓		✓	✓	✓		✓	✓	✓

blood tests as part of routine care											
Review of CT scans performed as part of routine clinical care (if available)	✓				✓						
BATT using ultrasound	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
BIA	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Handgrip strength	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Short Physical Performance Battery	✓			✓				✓	✓	✓	✓
Gait speed alone			✓				✓				
Physical function by PROMIS	✓		✓	✓	✓		✓	✓	✓	✓	✓
Katz ADLs and Lawton IADLs	✓		✓	✓	✓		✓	✓	✓	✓	✓
Mini Nutritional Assessment (Full)	✓			✓	✓			✓	✓		✓
Extra frailty assessments	✓		✓	✓	✓		✓	✓	✓	✓	✓
Venepuncture (optional)	✓	✓			✓	✓			✓		
Application of physical activity recorder (optional)		✓				✓			✓		
Delirium assessment		✓	✓		✓	✓	✓		✓	✓	
Fluid balance assessment		✓	✓		✓	✓	✓		✓	✓	
Participant feedback				✓				✓			✓

PROMIS = Patient Reported Outcome Measures Information System; ADLs = Activities of Daily Living; *Visit A: Preoperative assessment; in preoperative assessment clinic for elective cohort, on ward prior to surgery for emergency surgery cohort (where possible) – not applicable to medical cohort; Visit B: Immediate; Within 48 hours of surgery for surgical cohorts, within 48*

hours of admission for medical cohort; Visit C: One week; 7 (+/-2) days after surgery (surgical cohorts) or after admission (medical cohort); Visit D: Three months; 13 (+/-1) week after surgery (surgical cohorts) or admission (medical cohort)

2.1.3.4.1 Muscle quantity assessment

2.1.3.4.1.1 Quadriceps ultrasound

Rectus Femoris (RF) and Vastus Intermedius (VI) muscles in both legs will be assessed using two-dimensional B-mode ultrasonography with a linear probe, as previously described (Wilson et al., 2019). This will be performed at first visit, immediately postoperatively (where applicable), at seven-day follow-up, and at 13-week follow-up. Participants will be positioned semi-upright with knees resting at 10-20° and advised to relax their muscles. The distance from greater trochanter to knee lateral joint line will be recorded and a mark placed on the skin mid-way between the two points. Measurements will be taken in line horizontally with these marks. Contact gel will be applied. Muscle thickness will be measured with the probe in transverse position. Depth will be adjusted until the femur and overlying structures are visible. The probe will be positioned such that the widest area of the RF appears over the midpoint of the femur. Frozen images at this location will be taken with the probe held in maximal relaxation.

Thickness measurements of subcutaneous tissues (SC), RF, and VI in a vertical line will be recorded, not including the fascia. Three frozen images will be used for all patients; a further image will be taken if there is greater than 10% variability between measurements. The mean of each reading will be used for analysis. BATT will be calculated as total thickness of right VI

+ right RF + left VI + left RF. BATT: SC ratio (BATT-SCR) will be calculated as BATT divided by total thickness of right SC + left SC (Welch et al., 2019). Where possible, cross-sectional area of the right and left RF will be measured. All measurements will be performed by an investigator with training in taking these measurements. The reliability of BATT has been shown to be excellent when using the same protocol and same machine (intraclass coefficients > 0.9 for both intra-rater and inter-rater variability) (Wilson et al., 2019).

A further image will be taken in the longitudinal position at each visit. Images will be saved and downloaded for assessment. RF and SC echogenicity will be determined using grey-scale analysis on Image J software. Pennation angle will be measured by the angle of insertion of the fascicles within the VI to the deep aponeurosis. The mean measure from up to three fascicles measured on each image will be used for analysis (Strasser et al., 2013).

2.1.3.4.1.2 Bioelectrical Impedance Analysis (BIA)

BIA measurements will be taken at the first visit, immediately postoperatively (where applicable), at seven-day follow-up, and at 13-week follow-up using a multi-frequency analyser; Bodystat Quadscan 4000. This will not be performed if the participant has an implanted permanent pacemaker or defibrillator. The participant will be positioned lying semi-upright with knees resting in extension on the examination couch, hospital bed or equivalent. The assessor will ensure that limbs are not touching. Two electrodes will be placed on the right foot; one below the base of the toes and the other on the ankle between the medial and lateral malleoli. The red alligator clip will be attached to the electrode nearest the

toes and the black to the one at the ankle. A further two electrodes will be placed on the right hand; one behind the knuckles and the other on the wrist next to the ulnar head. The red alligator clip will be attached to the electrode nearest the fingers and the black to the one at the wrist. Electrodes will be placed transversely so that the non-stick electrode connector is facing the researcher. The Bodystat Quadscan 4000 includes a quality control feature; an impedance graph is displayed prior to results being displayed. If the graph shows a smooth curve, the investigator will proceed to record results. If there are any bumps in the graph, the investigator will recheck lead and limb position prior to repeating the analysis. Single measurements will be recorded at each visit.

All returned measures including prediction marker, impedance, resistance, reactance, phase angle, fat weight, lean weight, dry lean weight, Fat Free Mass Index (FFMI), Body Fat Mass Index (BFMI), total body water, extracellular water, and intracellular water will be recorded. Skeletal muscle mass (SMM) will be additionally estimated using three previously validated equations: 1) SMM = 0.566 x Fat Free Mass (lean mass) (Bahat et al., 2016); 2) SMM = [((height^2/ resistance) x 0.401) + (sex x 3.825) + (age x -0.071)] + 5.102. In the second equation, height is in cm, for sex male=1, female=0, and age is in years (Janssen et al., 2000). 3) SMM = -3.964 + (0.227 x (height^2/ resistance)) + (0.095 x weight) + (1.384 x sex) + (0.064 x reactance) (Sergi et al., 2015). For all equations, the skeletal muscle index (SMI) will be calculated through the formula SMI = SMM/ height^2, where height is in m, for comparison with normative populations (Janssen et al., 2004). Height and weight are recorded for all patients at the site of this study as part of routine clinical care. For the elective cohort, height will be measured in preoperative assessment clinic using a stadiometer. For the emergency surgery and medical

cohorts, height will be recorded using a stadiometer where possible. Where this is not possible, height will be taken from previous clinical records if these are available, or from patient report. If none of these methods are possible, then height may be estimated by measuring ulna length and conversion as per British Association of Parenteral and Enteral Nutrition guidelines (British Association of Parenteral and Enteral Nutrition, 2011). If an estimate has been used, this will be recorded. The same height will be used for all visits.

2.1.3.4.1.3 L3-CT using imaging performed during routine medical care

CT scans will be reviewed if these have been performed as part of routine care and are available. Skeletal muscle index will be calculated at the level of the third lumbar vertebra (L3) on the first image with both vertebral spines visible using local hospital site Picture Archiving and Communication Software. This will be calculated by manually identifying skeletal muscles and automatic calculation of cross-sectional area; this value will be corrected for height^2 (van der Werf et al., 2018). Total psoas area (TPA) will also be calculated on the same slice. The right and left psoas muscle borders will be manually outlined and TPA will be calculated within the selected area. This measurement will be corrected for height^2 (Hervochon et al., 2017). This will be performed by the investigating geriatrician who is trained in use of the software.

2.1.3.4.2 Muscle function assessment

2.1.3.4.2.1 Muscle strength

Handgrip strength will be measured at first visit, immediately postoperatively (where applicable), at seven-day follow-up, and at 13-week follow-up using a Jamar handheld dynamometer. Where the participant can sit in a chair, handgrip strength will be measured with the elbow flexed at 90^{O} and the forearm supinated. If measurements are taken in the bed this will be recorded; measurements will instead be performed in the most feasible upright position. Participants will be asked to "squeeze as hard as [they] can". Handgrip strength will be measured twice on each side and the highest recording of the four measurements will be used for analysis (Roberts et al., 2011).

2.1.3.4.2.2 Physical performance

The Short Physical Performance Battery (SPPB) is a standardised measure of physical performance that has been shown to be sensitive to change and provides an objective measure of physical function (Guralnik et al., 1994). SPPB consists of usual gait speed, side-by-side stand, semi-tandem stand, tandem stand, and five chair stands (as quickly as they can). A total score of 12 is derived, with a lower score representing reduced physical performance. The SPPB will be measured at baseline and three-month follow-up for the elective cohort, at three-month follow-up for the emergency surgery cohort, and at all visits for the medical cohort. Gait speed alone will be measured at seven-day follow-up for both

surgical cohorts. Gait speed will be measured by asking the participants to walk a four-metre course at their "usual pace". Gait speed will not be performed at recruitment in the emergency surgery cohort as this is considered unfeasible due to pain and immediate operative recovery. Measuring chair stands at one week post-operatively would cause increased abdominal strain, therefore, gait speed alone will be measured at this timepoint.

2.1.3.4.3 Physical function – Patient Reported Outcome Measures Information System (PROMIS®)

PROMIS physical function is a validated measure of physical function (Yost et al., 2011, Tatsuoka et al., 2016). PROMIS is an initiative that compares participant responses to a reference population and derives a T-score, where 50 is the mean, and 10 is the standard deviation. PROMIS will be measured at baseline and three-month follow-up for all groups. The raw scores will be entered into the HealthMeasures scoring service, powered by Assessment Center^{SM} to derive T-scores.

2.1.3.4.4 Comprehensive geriatric assessment

2.1.3.4.4.1 Demographics and comorbidities

Participant demographics, observations, medications, medical history, smoking and alcohol history, and blood test results performed as part of routine care will be collected. Medical comorbidities will be used to derive the Geriatric Index of Comorbidity (Zekry et al., 2010).

Number of admissions and falls over the previous year will be recorded. Falls will be recorded from participant report. Any new information including changes in weight, observations, medications, or blood tests will be recorded at each visit, dependent on when these are recorded as part of usual clinical care.

2.1.3.4.4.2 Nutritional assessment

The Mini-Nutritional Assessment (MNA®) is a validated assessment tool for nutritional status (Vellas et al., 1999). Much of the information required will be collected elsewhere. Additional information that will be collected will include food intake, specifically protein and fruit or vegetable intake, and fluid intake from participant report. Mid-arm circumference will be measured at the mid-point between the olecranon and acromium. Calf circumference will be measured as the widest part of calf. These measurements will be taken for the dominant limb. The MNA (full form) will be assessed at baseline visits and 13-week follow-up visits for each group.

2.1.3.4.4.3 Frailty assessment

Frailty will be assessed at first visit, seven-day follow-up, and 13-week follow-up using the Frailty Index (FI) (Rockwood and Mitnitski, 2007), 9-point Clinical Frailty Scale (CFS) (Rockwood et al., 2005), and phenotype definition (Fried et al., 2001). Activities of Daily Living (ADLs) will be assessed using Katz (basic ADLs) (Katz et al., 1963) and Lawton (instrumental ADLs) (Lawton and Brody, 1969) tools. The phenotypic diagnosis of frailty will be made if the

participant meets three out of five criteria: low gait speed, low handgrip strength, weight loss, self-reported exhaustion or low physical activity. Cut-offs used in the original phenotype diagnosis will be used for gait speed, handgrip strength, and weight loss. Self-reported exhaustion will be defined if the participant answers “most of the time” or “all of the time” to how often over the last week they had felt that either “everything [they] did was an effort” or they “could not get going” (Fried et al., 2001). Physical activity will be defined through self-report by asking the participant if over the last three months they have performed no weight-bearing physical activity, been for a short walk once/ month or less, or spent more than four hours/ day sitting (Fairhall et al., 2008). The FI will be calculated by counting the total number of deficits present out of 36 defined criteria, and dividing by 36. These criteria have been adapted for secondary care use from those previously validated in a UK community setting to form the electronic frailty index (Appendix 8.1.1) (Clegg et al., 2016). The CFS will be determined by the investigating geriatrician after clinical review and after all other information has been collected. The investigating geriatrician will determine this immediately after reviewing the participant by considering ADLs, physical function, self-reported exhaustion, and symptomatic burden reported by the participant.

2.1.3.4.5 Delirium screening and assessment

Delirium will be diagnosed by the investigating geriatrician as per the Diagnostic and Statistical Manual of Mental Disorders 5 (American Psychiatric Association, 2013). Participants will first be screened for evidence of delirium at each visit using the Single Question in Delirium “Do you think this patient has been more confused lately?” (Sands et al.,

2010). The investigating geriatrician will review notes and ask staff caring for the participant, family members, and the participant themselves. The participant themselves will be specifically asked “Has anything strange been happening?”, such as experiencing hallucinations. Where possibility of delirium is raised upon screening or during other assessments, the investigating geriatrician will perform further assessments to formally diagnose delirium by testing attention by months of the year backwards (Meagher et al., 2015), consciousness by the Modified Richmond Agitation and Sedation Scale (Sessler et al., 2002), and cognition by the Abbreviated Mental Test Score (Appendix 8.1.2).

2.1.3.4.6 Fluid balance assessment

Fluid balance will be assessed and recorded during all visits during hospitalisation. Fluid balance will be assessed by clinical assessment, review of input/ output charts, and BIA measurements. Clinical assessment by the investigating geriatrician will include review of skin turgor, mucus membranes, oedema, Jugular Venous Pressure level, trends in observations e.g. blood pressure, and patient presentation. The overall fluid status will be recorded as hypovolaemic, euvolaemic, or hypervolaemic for the participant overall. However, if unilateral oedema is present in a single limb this will be recorded. BIA measurements of TBW, ECW, ICW, and third space water will be recorded separately and assessed against all other available information of fluid balance.

2.1.3.4.7 Other outcome data

Further routinely collected data that will be recorded will include (as applicable) the operation performed, peri-operative blood loss, type of post-operative analgesia (patient-controlled analgesia or epidural), postoperative complications, length of stay, discharge destination, other hospital admissions within the three-month follow-up period, histological diagnosis (cancer vs. not), and one-year mortality.

2.1.3.4.8 Participant feedback

There is no standardised assessment tool for measuring test acceptability. However, a multi-faceted construct of acceptability has been proposed, which reflects the extent to which people receiving a healthcare investigation or intervention consider it to be appropriate. This construct consists of the affective attitude of the individual, procedure burden, individual ethicality (individual value system), intervention coherence (participant understanding), opportunity costs, perceived effectiveness, and self-efficacy (confidence that they can perform the necessary behaviour) (Sekhon et al., 2017). Considering this construct, we have devised a questionnaire that assesses each of these aspects separately for muscle quantity (for both ultrasound and BIA), handgrip strength, and gait speed testing (Appendix 8.1.3). These four aspects have been chosen as these will be measured most frequently for all participants in this study and have potential for direct translation into clinical practice. This will be administered to all participants at their final visit.

2.1.3.4.9 Venepuncture (optional)

Blood samples will be taken using the BD vacutainer Safety-Lok™ system in sterile vacutainers without additives (BD biosciences). Samples will normally be taken peripherally but may be taken centrally or via arterial lines if these are in place as part of routine clinical care. Samples will be taken at first visit, and where possible, within 48 hours of surgery in the surgical cohorts. Blood samples will be centrifuged within 30 minutes to one hour of collection, within the University of Birmingham Research Laboratories, within QEHB. Serum and plasma samples will be removed using calibrated pipettes and stored at -80^{o}C prior to further analysis. Serum and/or plasma concentration levels of high sensitivity C-Reactive Protein (hsCRP), Dehydroepiandrosterone sulfate (DHEA-s), cortisol, 25-OH vitamin D, Interleukin 6 (IL-6), Tumour Necrosis Factor Alpha (TNF-α), and Insulin-like Growth Factor (IGF-1) will be measured using Enzyme-Linked Immunosorbent Assays or other appropriate tests. Further additional biomarkers may be tested as appropriate. Remaining serum and/or plasma samples will be stored for use in future ethically approved research within the University of Birmingham Research Laboratories.

2.1.3.5 Fitbit Inspire physical activity quantification (optional)

The Fitbit Inspire will be applied to the non-dominant wrist during hospitalisation where this is agreed by the participant or consultee. This will record activity statistics during hospitalisation including number of steps taken, distance travelled, and sedentary time.

Summary statistics will be recorded for up until 30 days after hospitalisation. Participants will be advised to wear the monitor all the time. They will be supplied with a charger and advised to charge the device every five days when at rest, such as at night time. Position changes (e.g. sit to stand) will not be specifically recorded with this device.

2.1.3.6 Statistical analysis

2.1.3.6.1 Power calculation

The sample size for this study has been calculated by considering estimates of the precision of outcomes; 80% power and 5% significance level have been used in calculating this sample size. Allowing for 25% loss to follow-up from a sample size of 56, based on a paired t-test, the following clinically important changes may be detected with a sample size of 45 in each group (all changes are powered to be bidirectional and may be identified at multiple timeframes):

- Change of 6 for t-score derived from physical function measured by PROMIS (Mean 50, SD = 10) – from baseline to one week and/or from baseline to 13 weeks. This is validated from previous studies (Yost et al., 2011).
- Change of 0.66cm in BATT (Mean 3.6cm, SD = 1.1cm) – from baseline to one week and/or from baseline to 13 weeks. This is consistent with clinical change detected in our pilot study (mean loss of 0.76cm) (Welch et al., 2019).
- Change of 0.6 in skeletal muscle mass index measured using BIA (Mean 8.5, SD = 1) – from baseline to one week and/or from baseline to 13 weeks. This is consistent

with change detected in previous studies, consistent with acute sarcopenia (Martone et al., 2017).

- Change of 6kg in handgrip strength (Mean 23, SD = 10) – from baseline to one week and/or from baseline to 13 weeks. This is validated from previous studies (Roberts et al., 2011).

2.1.3.6.2 Data analysis

Data analysis will be conducted using IBM SPSS® Version 22. Results for each cohort will be analysed separately, although secondary data analysis will be conducted on all groups together. Interim analysis is planned with involvement from a patient and public involvement panel. Outcomes will be summarised at baseline, 7 days postoperatively, and 13 weeks postoperatively. The data analysis of the primary research question will include the following models:

- Unadjusted model with PROMIS at 13 weeks as the outcome of interest, and the secondary outcome as the covariate of interest (i.e. change in BATT, handgrip strength and/or gait speed from baseline to 7 days).

- Adjusted model with PROMIS at 13 weeks as the outcome of interest, and the secondary outcome as the covariate of interest (i.e. change in BATT, handgrip strength and/or gait speed from baseline to 7 days), with adjustment for the baseline PROMIS score, and patient demographics (e.g. age, gender).

- Model with PROMIS at 13 weeks as the outcome of interest, with adjustment for baseline PROMIS, and all secondary outcomes as covariates to establish the strength of association between those secondary outcomes and the primary outcome. This model will be used to establish which of the secondary outcomes (i.e. change in BATT, handgrip strength and/or gait speed from baseline to 7 days) is most strongly associated with change in PROMIS at 13 weeks.

Change in PROMIS rather than change in SPPB, which can be considered an objective measure of physical function, has been selected as our primary outcome for two reasons. Firstly, our patient and public involvement panel considered their own perception of their physical function to be most important. Although perception of function may differ from objective function, how function is perceived for them as individuals was considered more important. Secondly, it will only be possible to obtain true pre-hospitalisation measures of SPPB for the elective cohort. PROMIS provides a method of evaluating physical function prior to admission in the emergency cohorts. Change in SPPB will be evaluated as a secondary outcome in the elective cohort.

2.1.3.6.3 Acute sarcopenia

Sarcopenia will be defined as per EWGSOP2 as handgrip strength below 16kg in women or below 27kg in men (Dodds et al., 2014, Cruz-Jentoft et al., 2018), in combination with low muscle quantity or quality. Cut-offs for low muscle quantity and quality will be evaluated comparing the cohort against reference data in healthy young adults; cut-offs for BATT of

3.85cm in women and 5.44cm in men have been proposed (Wilson et al., 2019). Severe sarcopenia will be defined as additional presence of low physical performance; gait speed 0.8m/s or less (Studenski et al., 2011) or SPPB of 8 or less (Pavasini et al., 2016). Acute sarcopenia will be defined as incident sarcopenia compared to baseline measurements at recruitment. The prevalence of sarcopenia will be calculated at each visit.

2.1.3.7 Patient and public involvement

Older adults have been involved in the design and development of this research. We will host further discussion groups when analysing the results (interim and final). This will be particularly valuable when determining the significance of unexpected results. The interim meeting will be of potential value in assessing if any protocol amendments are necessary. The third discussion group will also be used to co-produce the study report. The findings of this research will be disseminated to all participants and their advocates through a written summary. The participants who are enrolled in this study itself will be the best placed to assess and comment on the acceptability of the procedures used during this study. Within the study design itself, we have devised a questionnaire to derive a multi-faceted acceptability score for assessment of muscle quantity using ultrasound, handgrip strength, and walking speed. We consider that formally interviewing participants could lead to unnecessary burden, given the time they will have already dedicated to the study itself. However, any informal feedback given will be recorded to guide further research and healthcare policy. The final formal meeting that is planned with our discussion group will encompass a full evaluation of the importance of the results of this study and will be used to co-produce any protocols for future research, as well as recommendations for healthcare policy.

2.1.3.8 Trial registration

This study was registered on ClinicalTrials.gov (identifier: NCT03858192) on 28th February 2019.

2.1.4 Discussion

This study will fully characterise changes in muscle quantity and function in a clinical setting and will provide invaluable information to researchers, clinicians, and patients. The results of this study have potential to lead on directly to further interventional studies to counteract these changes, with particular focus on identified mechanistic associations and clinical factors to guide risk stratification. The results may also lead to direct changes in clinical practice, including the embedding of our research tools into clinical practice, and changes in policy, such as promoting early mobilisation. Providing patients and members of the public with increased knowledge on their risk of declines in muscle quantity and function, and what this is likely to mean for them, can help to empower them in their own decision making and engagement with treatment and therapy.

We recognise that there are a number of limitations of our study. Firstly, this is a single site study, and therefore, the results may not be generalisable to the wider population. Secondly, the cohorts we have included are disease-specific. However, we consider that our results will provide proof of concept, which can be used to guide further research to increase

understanding in other disease populations. As described, our study has been powered to detect within group differences in the minimally clinical important differences as derived from other studies. However, this has not been powered separately for gender and other covariates (e.g. cancer vs. not), which may affect measurements. Interim analysis has been planned and our patient and public involvement panel will be involved in the interpretation of these results. At this stage, we will review the overall progress of the study and consider if protocol amendments may be necessary.

There is some evidence that position can affect measurement of muscle quantity by ultrasound and BIA (Hacker et al., 2016, Slinde et al., 2003). However, the results will be compared to a reference group of young healthy individuals taken in the same position as we have described, using the same technique (Wilson et al., 2019). The position described is one that we can consider to be feasible for measurements in a variety of different clinical environments, whilst ensuring the quadriceps are relaxed. Particular care will be taken to standardise the position of each measure for each participant across separate visits. Nevertheless, we acknowledge that due to measurements being taken in different clinical environment, there may be small uncontrollable differences in position across visits.

Venepuncture and physical activity recording have both been included as optional aspects of the study, and it is not known what percentage of participants will agree to these. However, venepuncture was previously included as an optional aspect within our pilot study and all participants were in agreement with this (Welch et al., 2019). We recognise that there will be

limitations of physical activity measurements recorded through the Fitbit Inspire. These devices will be unable to specifically measure change in position (e.g. sit to stand). Previous studies using raw accelerometer data have shown a floor effect when measuring physical activity in frail, sedentary older adults (Heesch et al., 2018). However, physical activity measures using Fitbits have also shown to correlate well with raw accelerometer data in studies involving older adults (Straiton et al., 2018). The Fitbit Inspire is considered to be an acceptable device for older adults due to its simple wristwatch-like design, and their low cost means that they are potentially utilisable in clinical practice. Within our study, we will assess the feasibility of using these devices and assess the validity of data recorded as covariates and predictors of change in muscle parameters and physical function.

Despite these limitations, we consider the recruitment of a complex heterogeneous population to be a strength of this study. Frail older adults are frequently under-represented in research studies. It is not possible to be certain that changes seen in young healthy adults are concordant with changes in older adults. Determining the mechanisms involved in the development of acute sarcopenia will enable risk stratification, and targeted interventions to prevent or even reverse the effects.

2.1.5 Ethics approval and consent to participate

This research has been sponsored by and reviewed by the University of Birmingham research governance team. Ethical approval has been obtained from Wales REC 4 (19/WA/0036) and

the Health Research Authority. Written informed consent will be obtained from all participants who are considered to have capacity to consent for themselves. Written personal or professional consultee declaration will be obtained if the participant is considered to lack capacity to consent to participation.

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Chapter 2.2 - Effect of position and exercise on measurement of muscle quantity and quality: towards a standardised pragmatic protocol for clinical practice

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Author contributions: DW developed the original ultrasound protocol that was adapted for use in this study. CW, ZM, TAJ, VK, and ZH-S were all significantly involved in the design and development of this study. Recruitment to this study and data collection were performed by CW, ZM, CK, IA, and HP. CW conducted the main analysis of the results and manuscript preparation. All authors significantly contributed towards interpretation of the results and agreed the final submitted version of the manuscript.

2.2 Effect of position and exercise on measurement of muscle quantity and quality: towards a standardised pragmatic protocol for clinical practice

2.2.1 Abstract

Background: Ultrasonography is an emerging non-invasive bedside tool for muscle quantity/quality assessment; Bioelectrical Impedance Analysis (BIA) is an alternative non-invasive bedside measure of body composition, recommended for evaluation of sarcopenia in clinical practice. We set out to assess impact of position and exercise upon measures towards protocol standardisation.

Methods: Healthy volunteers aged 18 – 35 were recruited. Bilateral Anterior Thigh Thickness (BATT; rectus femoris and vastus intermedius), BATT: Subcutaneous Ratio (BATT:SCR), and rectus femoris echogenicity were measured using ultrasound and BIA was performed; 1) lying with upper body at 45° (Reclined), 2) lying fully supine at 180° (Supine), 3) sat in a chair with upper body at 90° (Sitting), and 4) after exercise Reclined. Variability of Skeletal Muscle Mass (SMM) by two different equations from BIA (SMM-Janssen, SMM-Sergi), phase angle, fat percentage, and total body (TBW), extracellular (ECW), and intracellular water (ICW) were assessed.

Results: Forty-four participants (52% female; mean 25.7 years-old (SD 5.0)) were recruited. BATT increased from Reclined to Sitting (+1.45cm, 1.27 – 1.63), and after exercise (+0.51, 0.29 – 0.73). Echogenicity reduced from Reclined to Sitting (-2.1, –3.9 – -0.26). SMM-Sergi declined from Reclined to Supine (-0.65kg, -1.08 – -0.23) and after exercise (-0.70kg, -1.27 – -0.14).

ECW increased from Reclined to Sitting (+1.19L, 0.04 – 2.35). There were no other statistically significant changes.

Conclusion: Standardisation of protocols is especially important for assessment of muscle quantity by ultrasonography; BIA measurements may also vary dependent on the equations used. Where possible, participants should be rested prior to muscle ultrasonography and BIA, and flexion of the knees should be avoided.

2.2.2 Background

Sarcopenia is a condition of increasingly recognised significance in research and clinical practice. It is defined as reduced muscle strength with reduced muscle quality and/or quantity, and associated with significant detriments in quality of life and adverse health outcomes (Cruz-Jentoft et al., 2019). Dual-energy X-ray Absorptiometry (DXA), Computed Tomography (CT), and Magnetic Resonance Imaging (MRI) are recommended as Gold Standard for muscle quantity measurement (Cruz-Jentoft et al., 2019), but these are time-consuming, cannot be performed at the bedside, and are rarely performed serially. Ultrasonography is an emerging tool for assessment of muscle quantity and quality as part of evaluation for sarcopenia (Perkisas et al., 2018, Wilson et al., 2019, Ticinesi et al., 2017). It has evident benefits in that it is non-invasive, without exposure to ionising radiation, and provides point of care measurement in a number of settings. However, there is a lack of agreement on how muscle ultrasonography protocols for sarcopenia assessment should be standardised across clinical settings, including participant position and rest requirements pre-procedure (Perkisas et al., 2019, Ticinesi et al., 2019). Bioelectrical impedance analysis (BIA) is an

alternative safe technique for assessment of muscle quantity; phase angle, a direct measure of the angle between resistive current and total current, has been proposed as a measure of muscle quality by BIA (Norman et al., 2012). Higher values suggest greater cellularity and cell membrane integrity. The use of BIA has been criticised in research settings, due to reduced accuracy compared to DXA, CT, and MRI (Buckinx et al., 2018). However, it may be a pragmatic tool in clinical practice for body composition estimation (Cruz-Jentoft et al., 2018); it can be performed within minutes at the bedside, with minimal training. This study set out to evaluate the effect of changes in position and exercise upon muscle quality and quantity measured using ultrasound and BIA, in order to demonstrate the validity and recommendations of either or both techniques for use in clinical practice.

2.2.3 Methods

2.2.3.1 Participants

Healthy young adults aged 18 to 35 were recruited to this study at the University of Birmingham Research Laboratories, Queen Elizabeth Hospital Birmingham, in February 2020. Ethical approval was obtained from the University of Birmingham Science, Technology, Engineering and Mathematics Ethical Review Committee (ERN_19-1173). All study participants provided written informed consent to participate in this study. Exclusion criteria were: acute or chronic infectious or inflammatory conditions, inability to mobilise independently without walking aids, and the use of immunosuppressive agents or systemic steroids. Data were collected on age, sex, ethnicity, and physical activity via the Global

Physical Activity Questionnaire (Armstrong and Bull, 2006). Handgrip strength, gait speed over four metre course, height, and weight were measured for all participants.

2.2.3.2 Ultrasonography

Bilateral Anterior Thigh Thickness (BATT) was measured as described previously (Wilson et al., 2019) with B-mode ultrasonography using a linear probe (Venue 50, GE Healthcare). A mark was made on the skin at the midpoint between the greater trochanter and the lateral joint line of the knee on both sides and all measurements were taken at this mark. Participants were advised to relax their muscles. Contact gel was applied to the skin. The rectus femoris (RF) was identified by locating its border, and the probe was positioned in the transverse plane so that the RF was central over the femur. The thickness of subcutaneous tissue (SC), RF, and vastus intermedius (VI) were measured in real time at central point of greatest thickness, with the probe held in maximal relaxation, to a depth of 7cm. If it was not possible to view the entire VI, the minimum visible thickness was used in analysis. A minimum of three measurements were taken on each side; a fourth was taken if measurements differed by more than 10%. The mean of all measurements on each side was calculated and used in analysis. Bilateral Anterior Thigh Thickness was calculated as the total of right (RF + VI) + left (RF + VI). BATT:SC ratio (BATT:SCR) was calculated by dividing BATT by total bilateral SC. This method has been shown to have excellent intra-rater and inter-rater variability when using the same protocol (Wilson et al., 2019). All images for individual participants were taken by the same sonographer. All images were saved and remotely checked by a second experienced sonographer to ensure satisfactory views and measurements had been obtained. A further

image was taken in the longitudinal plane and RF grey scale analysis was performed using Image J software (Wilson et al., 2019). Grey scale analysis was calculated by drawing a square within the RF and analysing within this section. A measure of between 0 (black) and 255 (white) was returned. Echogenicity was calculated as the mean RF grey scale from both sides. This provides a measure of muscle quality and is considered to correlate with intramuscular fat infiltration (Wilson et al., 2019).

2.2.3.3 Bioelectrical Impedance Analysis

Impedance was measured using a Bodystat Quadscan 4000. Electrodes were placed on the right hand and foot as per the manufacturer's instructions and connected to the device. Height, weight, and age were inputted into the device and readings were then generated. All readings were recorded in real time including impedance, resistance, reactance, phase angle, fat percentage, total body water (TBW), extracellular water (ECW), and intracellular water (ICW). These are readings that are provided directly from the device using internal calculations. The phase angle equation is shown in Table 1. Skeletal muscle mass (SMM) was calculated using two widely accepted calculations – SMM-Janssen (Janssen et al., 2000) and SMM-Sergi (Sergi et al., 2015), as shown in Table 2.2-1.

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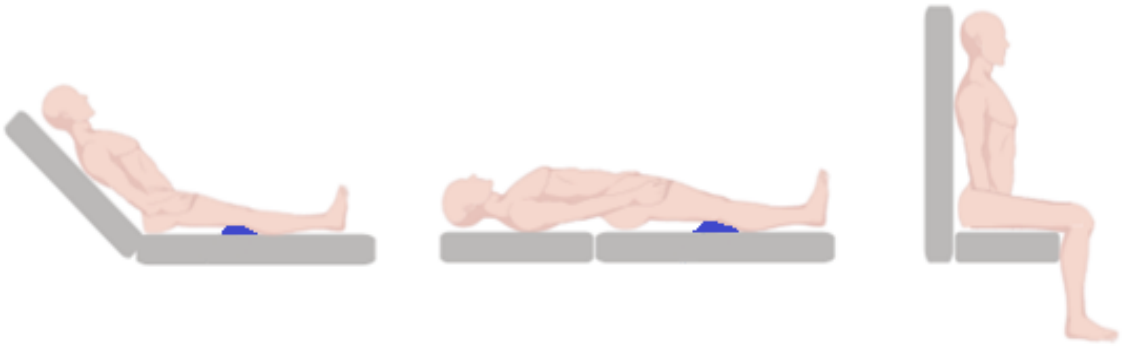
Table 2.2-1 – Equations used in calculation of Skeletal Muscle Mass (SMM) using bioelectrical impedance analysis.

In both equations: Height in cm; Sex 1=male, 0=female; Weight in kg; Resistance in Ω; Reactance in Ω

Skeletal Muscle Parameter	Equation
SMM-Sergi (Sergi et al., 2015)	= -3.964 + [0.227 × (height^2/resistance)] + (0.095 × weight) + (1.384 × Sex) + (0.064 × reactance)
SMM-Janssen (Janssen et al., 2000)	= [(height^2/resistance) × 0.401] + (Sex × 0.3825) + (Age × -0.071) + 5.102
Phase angle	= arctan(reactance/resistance) *"arctan" is the inverse trigonomic function (arc tangent) of the tangent function*

2.2.3.4 Positions and exercise protocol

Initial BIA and ultrasound measurements were taken following a period of rest with the participant positioned lying on a couch, with their upper body at 45^o and a firm wedge placed below their knees (Reclined). Measurements were then repeated with the participant lying flat at 180^o with the same wedge (Supine), and sat in a chair at 90^o (Sitting). The chair and couch used were of a similar firmness. Participants were advised to complete 20 star-jumps, 20 squats, and 20 burpees, or until they tired (Appendix 8.2.1). Measurements were then repeated immediately in the Reclined position. Figure 1 shows the different positions that were utilised. The same order of measurements was used for all participants.



Reclined **Supine** **Sitting**

Figure 2.2-1 – Positions utilised during study.

In the Reclined position participants were positioned at 45° with a wedge below their knees, in the Supine position participants were positioned supine with a wedge below their knees. In the Sitting position participants were sat upright in a chair.

2.2.3.5 Statistical analysis

Data were imported into IBM SPSS Statistics 26 for analysis. Descriptive statistics are represented in text and tables. Normalities of outcomes were assessed visually using Q-Q plots and histograms, and statistically by Shapiro-Wilk tests. Where outcomes were normally distributed, differences across positions and after exercise were assessed using linear mixed models to account for missing data. If not normally distributed, differences were assessed using generalised linear mixed models.

2.2.4 Results

2.2.4.1 Participants

Forty-four participants were recruited; mean age 25.7 (SD 5.0), 52% female. Full participant characteristics are shown in

Table 2.2-2. Missing data and participant inclusion for each position, including data excluded on second review, are shown in the online supplement (Appendix 8.2.2).

Table 2.2-2 – Participant characteristics

		Study population (N=45)
Age (years) – mean (SD)		25.7 (5.0)
Gender – Female % (N)		52% (23)
Ethnicity	Black or Black Mixed % (N)	34% (15)
	East Asian or Mixed East Asian % (N)	14% (6)
	South Asian or Mixed South Asian % (N)	52% (23)
METminutes/week – mean (SD)		3436 (2790)
Sedentary minutes/week – mean (SD)		2876 (1308)
Meeting recommended activity – % (N)		98 (43)
Body Mass Index (kg/m^2) – mean (SD)		23.4 (4.0)
Handgrip strength (kg) – mean (SD)	Males	56.8 (12.5)
	Females	32.3 (4.6)
Gait speed (m/s) – mean (SD)		1.38 (0.26)

METminute = Metabolic Equivalent minutes METminutes were calculated from the Global Physical Activity Questionnaire as the sum of weekly vigorous (minutes × 8) and moderate (minutes × 4) activities performed as part of work, commuting, and leisure. Physical activity

cut-off of <600 METminutes/week was considered as not meeting recommendations (Armstrong and Bull, 2006).

2.2.4.2 Ultrasonography

2.2.4.2.1 Bilateral Anterior Thigh Thickness (BATT)

The RF and VI were measured in all patients in Reclined and Supine positions. However, the VI could not be fully visualised Sitting in 9.8% (4/41) of participants; in these cases, BATT was calculated from the visible VI thickness. Bilateral Anterior Thigh Thickness increased from Reclined to Sitting (+1.44 cm, 1.27 – 1.63; p<0.001), and after exercise (+0.51, 0.29 – 0.73; p<0.001) (Figure 2.2-2a). There was no statistically significant change from Reclined to Supine. Variations in individual participant data are shown in the online supplement (Appendix 8.2.3).

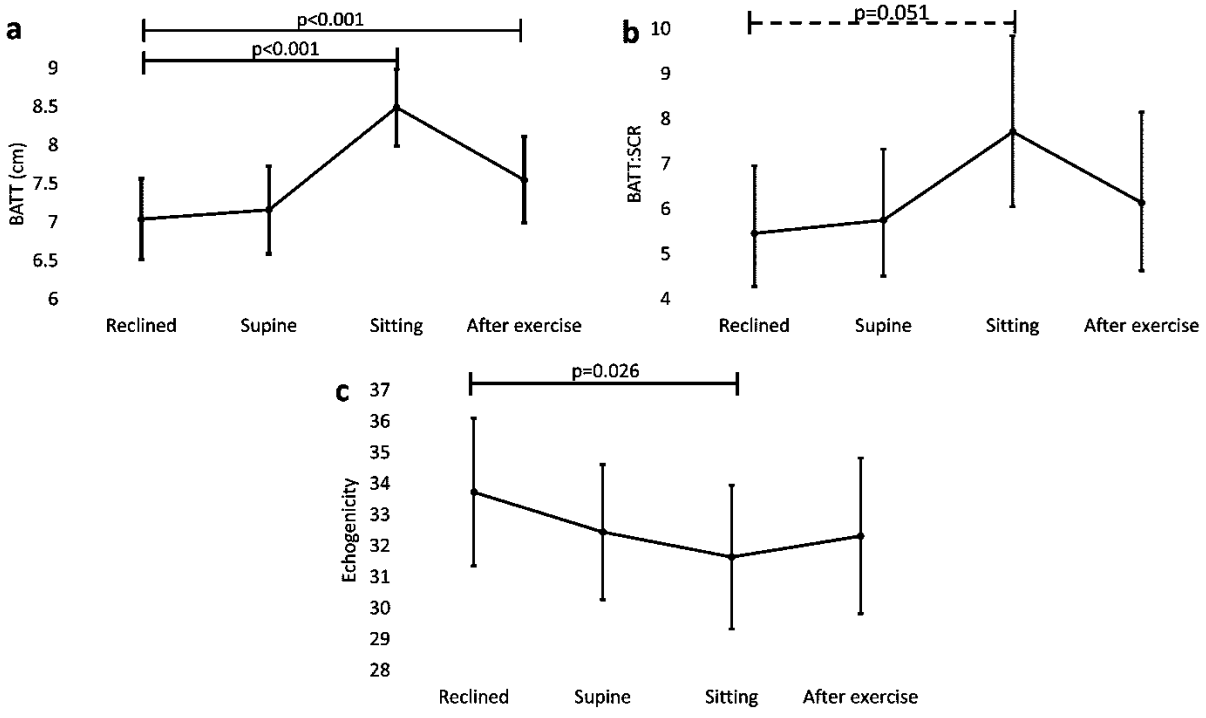


Figure 2.2-2 – Differences in ultrasonography measures between positions and after exercise.

Markers correspond to estimated means calculated from linear mixed models/ generalised linear models. Error bars correspond to 95% confidence intervals. *BATT=Bilateral Anterior Thigh Thickness; BATT:SCR=Bilateral Anterior Thigh Thickness: Subcutaneous tissue Ratio*

2.2.4.2.2 Bilateral Anterior Thigh Thickness: Subcutaneous Ratio (BATT:SCR)

Bilateral Anterior Thigh Thickness: Subcutaneous tissue Ratio did not significantly differ between positions but there was a trend towards decline from Reclined to Sitting (-2.26, -4.53 – +0.01; p=0.051) (Figure 2.2-2b).

2.2.4.2.3 Echogenicity

Echogenicity reduced from Reclined to Sitting (-2.1, CI -3.9 – -0.3; p=0.026), but other changes were not statistically significant (Figure 2.2-2c).

2.2.4.3 Bioelectrical Impedance Analysis

2.2.4.3.1 Skeletal Muscle Mass

SMM-Janssen did not differ significantly between positions or after exercise (Figure 3a). SMM-Sergi reduced from Reclined to Supine (-0.65, CI -1.08 – -0.23; p=0.004) and after exercise (-0.70, CI -1.27 – -0.14; p=0.016) (Figure 2.2-3b).

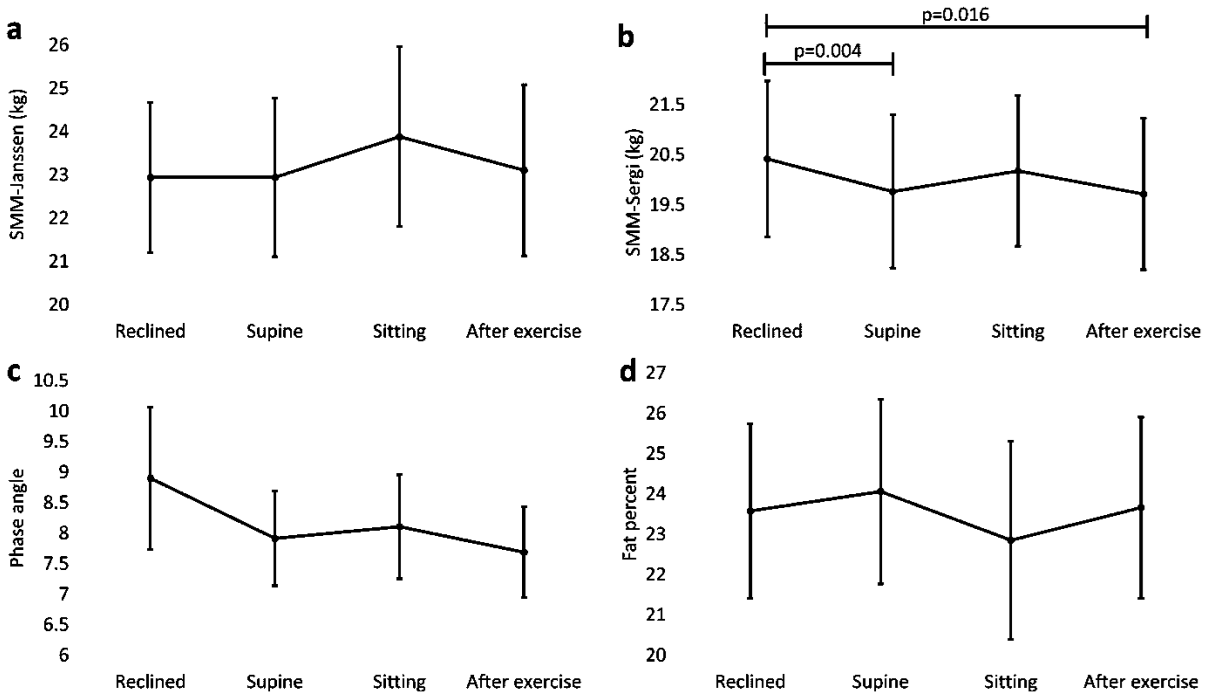


Figure 2.2-3 – Differences in muscle and fat measures by bioelectrical impedance analysis before and after exercise.

Markers correspond to estimated means calculated from linear mixed models/ generalised linear models. Error bars correspond to 95% confidence intervals. *SMM-Janssen=Skeletal Muscle Mass by Janssen equation; SMM-Sergi=Skeletal Muscle Mass by Sergi equation*

2.2.4.3.2 Phase angle

Phase angle did not statistically significantly differ between positions or after exercise (Figure 2.2-3c).

2.2.4.3.3 Fat percentage

Fat percentage did not differ significantly between positions or after exercise (Figure 2.2-3d).

2.2.4.3.4 Water distribution

TBW, ECW, and ICW did not significantly differ between positions or after exercise (Figure 2.2-4a-c).

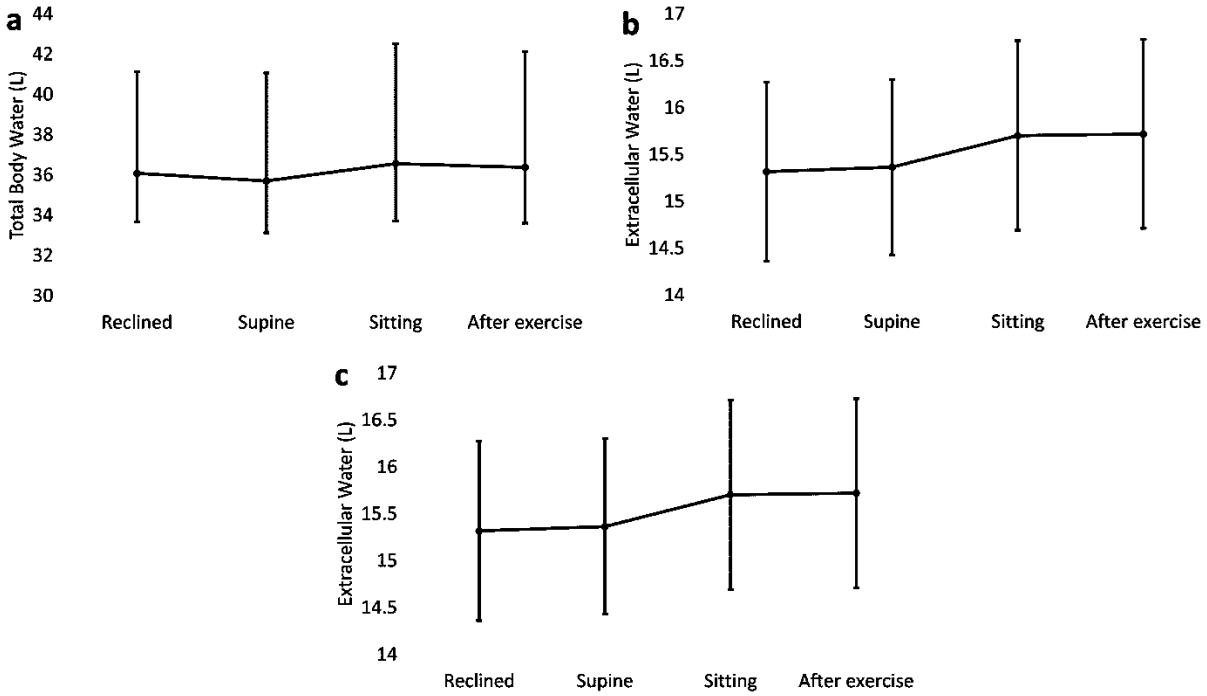


Figure 2.2-4 – Differences in water distribution measurements by bioelectrical impedance analysis.

Markers correspond to estimated means calculated from linear mixed models/ generalised linear models. Error bars correspond to 95% confidence intervals.

2.2.5 Discussion

2.2.5.1 Interpretation and implications for future research and clinical practice

Bilateral Anterior Thigh Thickness exhibited the greatest variance in relation to both position and exercise, the greatest of which was the effect of sitting in a chair. Increases in BATT were exhibited when participants were sat in a chair as compared to measurements performed on the examination couch. This is consistent with previous studies, which have shown increased

RF cross-sectional area in the seated position compared to supine (Tomko et al., 2018). As it was not possible to view the entire VI in all participants in this position, the demonstrated effect is likely to be an underestimate and the true difference may be even greater. To a lesser degree, BATT also increased after exercise. The exercise protocol used in this study was more intensive than a typical exercise protocol that might be used in a frail or hospitalised population. However, even small increments in physical activity could be equivalently demanding in people with frailty or acute illness; for an older frail person this could be simply the demand of walking across a room and getting onto an examination couch. Bilateral Anterior Thigh Thickness has been shown to have excellent intra-rater, inter-rater variability when using the same protocol (i.e. repeated measures in the same position); we are confident that these changes relate to the effect of position and exercise. Additionally, validity of measurements was ensured by review of all by a second experienced sonographer, including correct orientation of the RF over the femur.

Importantly, the difference in BATT between the recumbent and sitting positions (+1.44 cm) was greater than differences that have been observed in clinical studies measuring changes in muscle quantity in hospitalised populations (Welch et al., 2019) i.e. highly clinically significant. This difference is also greater than the 95% confidence intervals for estimated mean BATT in all positions. It is important to consider that the differences in BATT do not relate to true differences in muscle quantity within these short time frames. Increased BATT in the seated position likely relates to contraction and shortening of the RF with combined knee and hip flexion, leading to a greater cross-sectional area; the RF inserts at both the hip and knee joint (Tomko et al., 2018). As it is not possible to measure muscle volume with

ultrasonography, this emphasises why standardisation of protocols is vitally important. Similarly, BATT increased after exercise, likely related to persistent contraction of the quadriceps muscles. During exercise, metabolic requirements of skeletal muscles are increased and blood flow increases (Joyner and Casey, 2015). This in turn increases the temperature of muscles and reduces stiffness, promoting increased muscle activity i.e. muscle contraction in the neutral position.

Echogenicity declined in the seated position, but there were no significant changes after exercise. Additionally, the change in the seated position was smaller and potentially of less clinical significance. Echogenicity provides a numerical measure of muscle quality, which has been shown to correlate with muscle function (Wilson et al., 2019). Therefore, echogenicity may provide a more readily standardisable measure across settings, where standardisation of exercise protocols is challenging. However, echogenicity has been shown to exhibit greater inter-user variability compared to BATT (Wilson et al., 2019). As all images for individual participants were obtained by the same sonographer, this should not have affected changes demonstrated across repeated measures for individual participants.

As much as possible, position should be standardised when performing quadriceps muscle ultrasonography; where there are any deviations in position, these should be noted. The seated position may represent an option as a pragmatic, easily standardised position. However, as we were unable to obtain thickness measurements in all patients in this position, this may be less feasible without readily available machines/probes that measure to greater

depth. This is important when measuring healthy young adults as part of a reference standard, but may also be particularly relevant in individuals with increased subcutaneous tissue e.g. sarcopenic obesity. We recommend that ultrasonography measures should be taken with the knee in natural relaxation. As we did not find any clinically or statistically significant difference between the supine and 45^{o} positions, small variations in the tilt of the head of the bed can be tolerated, provided significant flexion of the knee is avoided.

Less variance was exhibited with BIA. Phase angle, SMM-Janssen, fat percentage, TBW, ECW, and ICW did not vary across any repeated measures statistically significantly. There were reductions in SMM-Sergi from the 45^{o} position to fully supine and after exercise. Pragmatically, this means that BIA can be performed in a variety of clinical settings, including where it is not practical to perform supine e.g. in a frail older person attending a clinic appointment in a wheelchair. A more reliable formula where the position of the upper body cannot be standardised but the patient/participant is able to lie on a couch or a period of rest prior to assessment is not feasible may be SMM-Janssen. Historically, BIA has been extensively criticised previously compared to DXA, CT or MRI in research settings, due to reduced precision (Buckinx et al., 2018). However, it is also important to consider the purpose of measuring muscle quantity and the degree of certainty that is necessary in clinical practice. BIA may be a pragmatic tool for screening and as an adjunct as part of a Comprehensive Geriatric Assessment. As well as less variability demonstrated in this study with positions and exercise, BIA is also much quicker to perform than ultrasound and requires minimal training. The phase angle has been proposed as a measure of muscle quality, as a measure of cell membrane function (Norman et al., 2012). However, BIA is known to be affected by fluid

balance (Ticinesi et al., 2017), although as technology and datasets develop it may be possible to perform correction calculations for this. BIA is also currently contraindicated in people with implanted cardiac devices; there is increasing evidence that it is likely to be safe (Chabin et al., 2019), but it is unknown if results can be reliably interpreted.

2.2.5.2 What are the limitations of this research?

Importantly, this research was performed in healthy young volunteers. Whilst our results provide preliminary results towards standardisation of a protocol for muscle quantity assessment, we recognise that results may be different in an older and/or hospitalised population. In older adults with sarcopenia, less variability in measures may be seen if muscles are already very small and insufficient. Indeed, a pragmatic interpretation may be that if muscle quantity is demonstrated to be reduced in the seated position, then it is very likely to be reduced in any other position. However, if muscle quantity appears normal it may still be reduced if measured without the hip and knee in combined flexion.

Conversely, in hospitalised populations it is plausible that greater variability in measures may be exhibited due to greater fluid shifts. This may affect measurements taken using ultrasonography as well as BIA. In our study, all participants were young, healthy, and clinically euvolaemic. There was no clinical evidence of change in hydration status between repeated measures, and hydration status measured by BIA itself also did not change with position. Additionally, nearly all participants were sufficiently physically active to meet the minimum World Health Organization (WHO) guidelines, which may have affected the responsiveness of

skeletal muscles to the effects of position and exercise. Our study was not powered to examine differences of position and exercise effect between groups (e.g. gender, ethnicity, activity levels). However, since participant characteristics did not change between repeated assessments, this will not have affected our overall results.

Whilst we consider the changes in BATT and BATT:SCR not to be related to true changes in muscle quantity, we recognise that we did not measure muscle quantity using any gold standard techniques. Due to the nature of the study, it was also not possible to blind assessors to position. Additionally, considering the effects of exercise, this study only evaluated the effects of very short high intensity exercise; the effects of longer periods of exercise, or less intensive physical activity are unknown. We also acknowledge that we cannot rule out effects of moving between positions in the order used, as we did not use a counterbalance design.

2.2.6 Conclusion

Measured muscle quantity, but not quality, varied by ultrasonography with changes in position and after exercise in this study involving healthy young volunteers. Muscle quantity measurements using BIA were not affected by position or exercise. Further research evaluating these changes in older adults will be valuable. However, as cut-off values for the diagnosis of sarcopenia are developed from young healthy reference populations (Cruz-Jentoft et al., 2018), we consider it important to standardise technique in these populations to ensure measures taken in clinical populations are comparable.

We recommend that ultrasonography measures should be taken with patients/participants resting on a bed/couch with knees in natural extension. Whilst prolonged periods of rest may not be practical, patients/participants should avoid exertion immediately before muscle ultrasonography; we recommend measuring physical performance afterwards. When deciding on appropriate tools for assessment, it is important to consider the purpose for muscle quantity/quality measurements. For screening purposes, then BIA may be suitable. If the purpose is for more comprehensive evaluation, then ultrasonography and BIA can be performed together as part of a comprehensive assessment e.g. to test responsiveness to interventions.

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3 Acceptability and feasibility of acute sarcopenia assessment

Chapter 3.1 – Muscle quantity and function measurements are acceptable to older adults during and post- hospitalisation: results of a questionnaire-based study

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3.1 Muscle quantity and function measurements are acceptable to older adults during and post- hospitalisation: results of a questionnaire-based study

3.1.1 Abstract

Background: To evaluate the acceptability of handgrip strength, gait speed, quadriceps ultrasound, and Bioelectrical Impedance Analysis (BIA) to older adults conducted during and following hospitalisation

Methods: Questionnaire-based study conducted upon completion of prospective cohort study, with follow-up in either Queen Elizabeth Hospital Birmingham (QEHB), UK, or participant's own home following recent admission to QEHB. Outcome measures were acceptability as defined by total multi-domain score for each test (maximum score 35), and by frailty status.

Results: Forty adults aged 70 years and older admitted for emergency abdominal surgery, elective colorectal surgery, or acute bacterial infections (general medicine) participated. Handgrip strength (median 33, IQR 30 – 35; p=0.001), gait speed (median 32, IQR 30 – 35; p=0.002), ultrasound quadriceps (median 33, IQR 31 – 35; p=0.001), and BIA (median 33.5, IQR 31 – 35; p=0.001) were considered highly acceptable. Participants responded positively that they enjoyed participating in these tests, and considered these tests of importance. There was no difference in scores between tests (p=0.166). Individual total test scores did not differ between patients with and without frailty. Qualitative data are also presented on drivers for research participation.

Conclusions: Handgrip strength, gait speed, ultrasound quadriceps, and BIA are acceptable tests to older adults during and following hospitalisation. Our results may serve as standards when evaluating acceptability of other tests.

Study registration: Prospectively registered February 2019: https://clinicaltrials.gov/ct2/show/NCT03858192

3.1.2 Background

Acceptability is a complex construct, but it is acknowledged that this can affect patient adherence both in clinical practice and research. A construct for measurement of acceptability has been proposed consisting of affective attitude, burden, ethicality, intervention coherence, opportunity costs, perceived effectiveness, and self-efficacy (Sekhon et al., 2017). Sarcopenia is an area of increasing research and clinical interest. It is defined by the European Working Group on Sarcopenia 2 (EWGSOP2) as reduced skeletal muscle strength with reduced muscle quantity/quality; additional demonstration of low physical performance defines severe sarcopenia (Cruz-Jentoft et al., 2019). Cut-offs are taken as two standard deviations (SDs) below the mean of young healthy reference populations. Acute sarcopenia refers to acute decline in muscle quantity/quality and/or function leading to incident sarcopenia within six months, normally following a stressor event (Cruz-Jentoft et al., 2019, Welch et al., 2018). EWGSOP2 recommends measurement of handgrip strength for muscle strength, and either Dual-energy X-ray Absorptiometry (DXA) or Bioelectrical Impedance Analysis (BIA) for evaluation of muscle quantity in clinical environments (Cruz-Jentoft et al., 2019). Ultrasonography is a recognised emerging alternative to DXA and BIA

(Wilson et al., 2019). Muscle quality can also be evaluated by ultrasound echogenicity (Wilson et al., 2019), or the BIA-measured phase angle (Norman et al., 2012). EWGSOP2 recommends assessment of physical performance by Short Physical Performance Battery (SPPB), gait speed, Timed Up and Go (TUG), or 400m walk time (Cruz-Jentoft et al., 2017). However, the acceptability of these measures to patients or research participants has not been previously evaluated.

3.1.2.1 Objectives

To evaluate the acceptability of handgrip strength, gait speed, quadriceps ultrasonography, and BIA to patients, when measured as part of an observational study during and post-hospitalisation. The aim of the main study was to characterise acute sarcopenia in hospitalised older patients.

3.1.3 Methods

3.1.3.1 Participants

The main protocol for this study has been published elsewhere (Welch et al., 2020). Our reporting is consistent with Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines. Patients were recruited to one of three cohorts from the Queen Elizabeth Hospital Birmingham (QEHB) – general medical patients with infections, elective colorectal surgery, or emergency abdominal surgery. Inclusion criteria for each

cohort were aged 70 years and older and hospitalised (or expected to be hospitalised for the elective cohort) for an acute bacterial infection, major colorectal surgery procedure, or emergency abdominal surgery procedure. Exclusion criteria were the inability to understand verbal and written English, or imminently dying. Informed consent or personal consultee declaration was obtained for all participants. Medical patients were recruited within 48 hours of admission, emergency surgery patients were recruited pre-operatively or within 48 hours post-operatively, and elective surgery patients were recruited in pre-operative assessment clinic.

3.1.3.2 Study design

Quadriceps ultrasound, BIA, handgrip strength, and physical performance (either SPPB or gait speed alone depending on cohort and timing of assessment) were measured serially as part of this study. These were performed within 48 hours of admission/surgery, within one week of admission/surgery, and three months after admission/surgery. In the elective cohort, measurements were also performed prior to admission.

3.1.3.3 Outcome measures

Quadriceps ultrasound was performed anteriorly over both thighs at the midpoint between the greater trochanter, and the joint line of the knee. Participants were positioned with their knees in natural relaxation, with a firm wedge below the knees, and the upper body reclined to 45^{o}. Contact gel was applied to the skin and measurements were taken using a linear probe

using a Venue 50 device (GE Healthcare). A minimum of three measurements were taken on each side; a fourth was taken if rectus femoris (RF), vastus intermedius (VI), or subcutaneous (SC) measures varied by more than 10% between each other. These measures were used to calculate the Bilateral Anterior Thigh Thickness (BATT – right RF + left RF + right VI + left VI) (Wilson et al., 2019). BIA was performed in the same position by applying electrodes to the right hand and foot and recording measures using a Bodystat Quadscan 4000 as per the manufacturer's instructions. BIA was not performed in participants with implanted cardiac devices. Handgrip strength was measured using a Jamar dynamometer; participants sat in a chair with their elbow flexed at 90^{o} and advised to squeeze as hard as they could (Roberts et al., 2011). Two readings were taken on each side. Gait speed was measured over a 4m course; participants were advised to walk at a normal comfortable pace, using walking aids if necessary.

3.1.3.4 Frailty

Frailty was defined dichotomously (frail vs. non-frail) according to the phenotype definition (Fried et al, 2001) at the point of the completion of acceptability questionnaire. Frailty was defined as scoring three or greater of weight loss (recorded or self-report), low handgrip strength, low walking speed, self-reported exhaustion, or low physical activity, as detailed in the main study protocol (Welch et al., 2020).

3.1.3.5 Acceptability evaluation

An acceptability questionnaire was developed, as described in the main study protocol (Welch et al., 2020) and Appendix 8.1.3, which asked participants to state how highly they agreed with positive statements about seven different aspects of acceptability for each of handgrip strength, 4m gait speed, ultrasound quadriceps, and BIA (Table 3.1-1) (Sekohn et al., 2017). The questionnaire was completed by the same researcher who administered the muscle quantity and function assessments. We evaluated gait speed alone rather than SPPB to ensure consistency across cohorts, and prevent burden to participants from the acceptability evaluation. Responses were given using a Likert scale (1=strongly disagree, 2=disagree, 3=neither agree nor disagree, 4=agree, 5=strongly agree). Participants were also able to provide additional comments related to the study in general or any study-related procedures. This questionnaire was administered to all participants at the point of their three-month follow-up, in either their own home, or the Inflammation Research Facility, QEHB. Recruitment was paused due to the Coronavirus 2019 (COVID-19) pandemic and the protocol was later amended to remove in-person follow-up at three months, to reduce unnecessary contact with vulnerable participants. This sub-study includes participants who were recruited prior to this amendment.

Table 3.1-1 – Positive statements included in acceptability questionnaire and applicable domains.

Participants were asked to rate their agreement with these statements on a scale from 1=strongly disagree to 5=strongly agree

Acceptability domain	Statement
Affective attitude	I enjoyed participating in this test
Burden	Minimal effort was required to complete this test
Ethicality	This test was unobtrusive
Coherence	I understand how this test works and its importance
Opportunity costs	This test was not time-consuming
Perceived effectiveness	This test is likely to have a positive impact on patients
Self-efficacy	I felt confident that I could complete this test

3.1.3.6 Statistical analysis

Data were imported into IBM SPSS Version 26. Counts for each Likert score were derived and presented visually with horizontal bar charts. For each outcome measure, a total score was derived for all acceptability domains for each participant (minimum possible score 7, maximum possible score 35). Median total acceptability scores were calculated for each outcome. We used one-sample Kolmogorov-Smirnov normal tests to evaluate distributions of total acceptability scores for each outcome. We used the Friedman test to assess differences in total acceptability scores between outcome measures and Mann-Whitney U tests to assess for differences in individual total scores between those with and without frailty. The main study was powered for a different primary outcome. A post-hoc power calculation showed that a sample size of 40 was able to detect a difference in total acceptability score of 1.35, with 80% power and 5% alpha, assuming a null hypothesis median

score of 20 (i.e. neither agree nor disagree selected for all answers) and an expected normal distribution.

3.1.3.7 Qualitative analysis

Free text comments were transcribed by the researcher (CW) linked against their identifiable study number. The researcher (CW) familiarised themselves with the comments and identified emergent themes. Thematic analysis was conducted using an inductive approach, with no prespecified hypotheses of what data may arise from these comments. The participant details were linked to text after identification of emergent themes. Consensus agreements of themes was reached by researchers not involved in initial transcription and data reduction.

3.1.3.8 Public involvement

Patients and members of the public were extensively involved in the planning and development of the main study. The questionnaire used in this study was developed with direct involvement of healthy older adults. The results of this study itself will be of direct relevance to future studies and clinical practice involving the measures described.

3.1.4 Results

Sixty-four participants (24 elective surgery, 24 general medical, 16 emergency surgery) were recruited to the main study from May 2019 to March 2020.

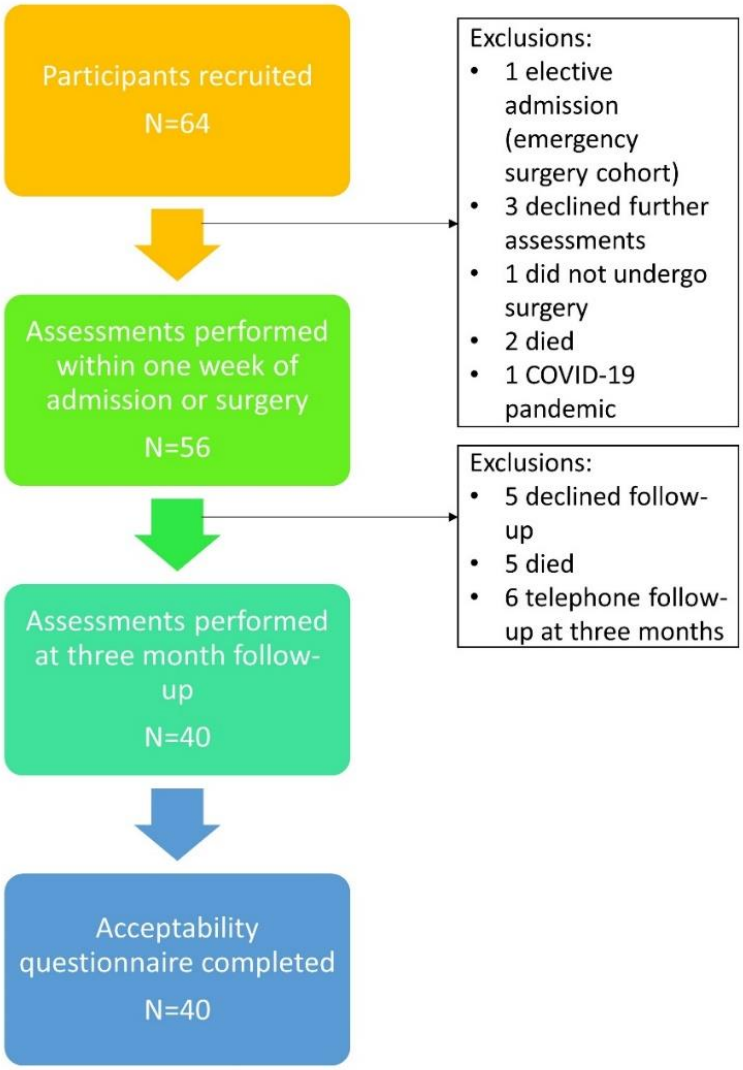

Figure 3.1-1 shows the recruitment and follow-up flowchart of included participants. Forty participants (17 elective surgery, 13 general medical, 10 emergency surgery) were followed-up in person at three months and all completed the acceptability questionnaire. The characteristics of participants who completed the questionnaire are shown in Table 3.1-2.

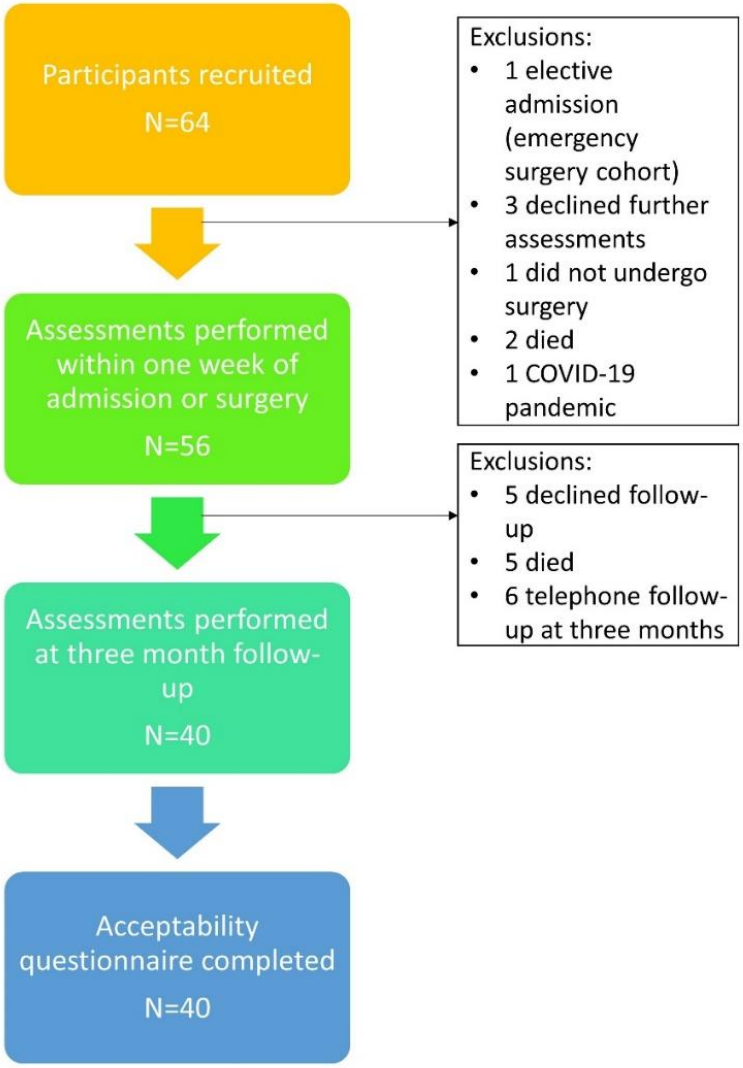

Figure 3.1-1 – Recruitment and follow-up of participants within main study.

Table 3.1-2 – Characteristics of participants who completed acceptability questionnaire

		Study population (N=40)
Age – mean (SD)		78.1 (6.3)
Sex – % females (N)		47.5 (19)
Ethnicity	White British or White Irish – % (N)	95.0 (38)
	Indian – % (N)	5.0 (2)
Phenotypic frailty at follow-up – % frail (N)		57.5 (23)
Gait speed – mean (SD)		0.67 (0.28)
	Males	25.8 (10.8)

Handgrip strength – mean (SD)	Females	16.9 (8.0)

3.1.4.1 Quantitative results

Figure 3.1-2 shows the distribution of response scores for each acceptability domain for each outcome measure. Overall, domains rated highly for all outcome measures, with the majority of participants stating that they agreed or strongly agreed with each positive statement for each outcome. The domain with the least agreeability was burden for both handgrip strength and gait speed; some participants disagreed with the statement that minimal effort was required to complete these tests. The domain with the highest agreeability was self-efficacy, particularly for ultrasound and BIA; participants agreed or strongly agreed that they enjoyed participating in these tests.

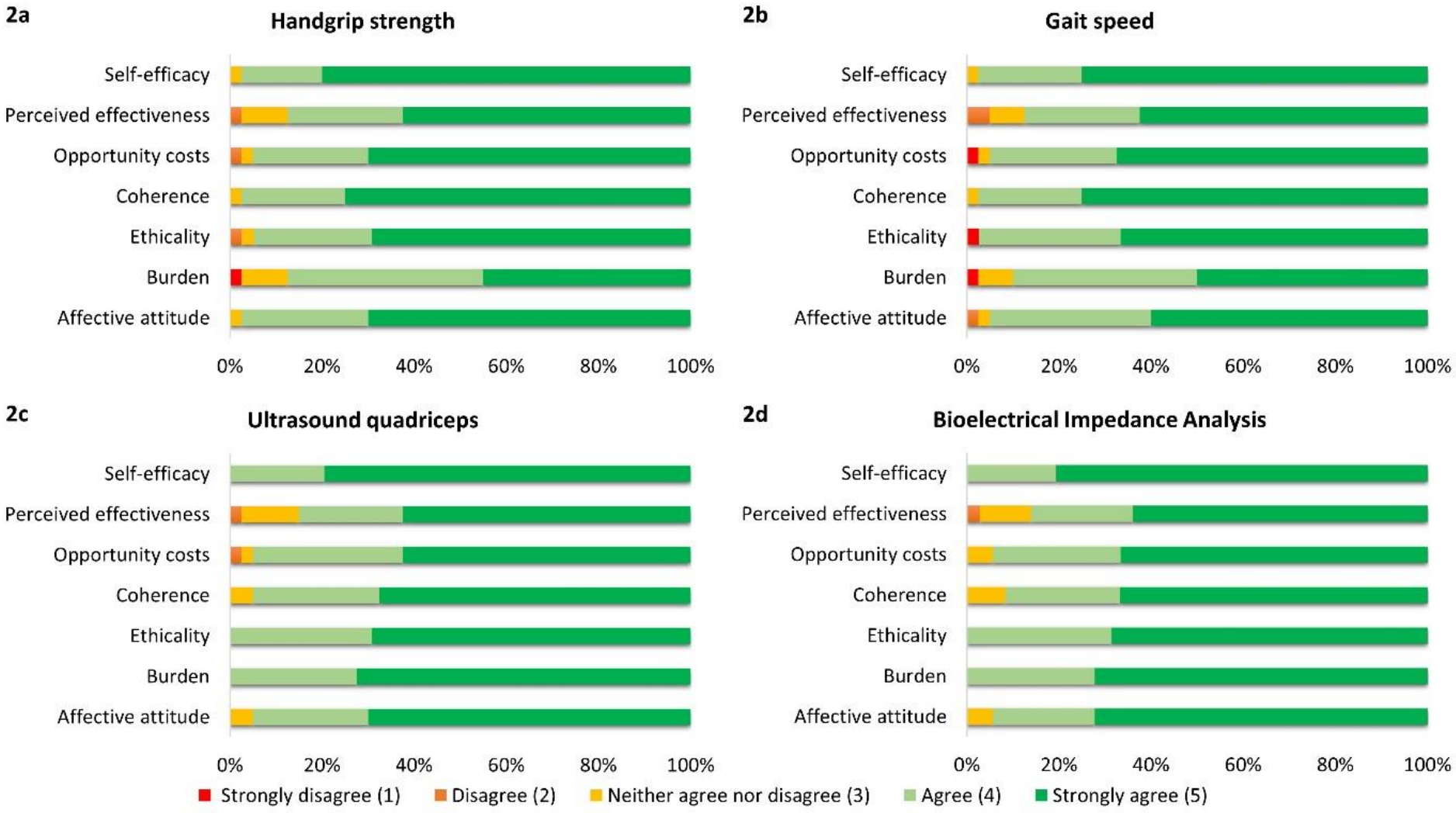

Figure 3.1-2 – Distributions of individual responses for each acceptability domain for each outcome measure

Table 3.1-3 shows the median overall scores for each outcome measure, separated by phenotypic frailty. All total score distributions were individually significant. However, total scores did not significantly differ between outcome measures. Additionally, scores did not significantly differ for outcomes between those with and without frailty.

Table 3.1-3 – Median test scores for total acceptability scores for each outcome measures overall and divided by phenotypic frailty status.
The minimum possible total median score was 7, and the maximum possible score was 35. Higher scores suggest higher levels of acceptability.

		Median (IQR)	p-value (one sample)	p-value (groups)
Overall				
Handgrip strength (N=40)		33 (30 – 35)	0.001	0.166
Gait speed (N=40)		32 (30 – 35)	0.002	
Ultrasound quadriceps (N=40)		33 (31 – 35)	0.001	
Bioelectrical Impedance Analysis (N=36)		33.5 (31 – 35)	0.001	
Frailty				
Handgrip strength	Frail (N=23)	33 (30 – 33)	0.052	0.396
	Non-frail (N=17)	34 (30 – 35)	0.030	
Gait speed	Frail (N=23)	32 (28 – 32)	0.031	0.242
	Non-frail (N=17)	34 (31 – 35)	0.019	
Ultrasound quadriceps	Frail (N=23)	32 (29 – 32)	0.042	0.386

	Non-frail (N=17)	34 (31 – 35)	0.008	
Bioelectrical Impedance Analysis	Frail (N=20)	32 (29 – 32)	0.009	0.352
	Non-frail (N=16)	34 (32 – 35)	0.008	

3.1.4.2 Qualitative results

3.1.4.2.1 Study procedures

Many participants commented positively on their experience of completing the study-related procedures. Some participants commented that they enjoyed completing the tests, in that they gave them something new to try, and additional knowledge about their health.

"I was looking forward to it actually [gait speed]; I thought at least it would get me moving…"

"Actually, I enjoyed doing all the tests"

"It was welcoming really to try to do things that I couldn't do 12 months ago"

"Anything positive to do with your health is definitely a good thing"

"It was quite relaxing"

"I'm pleased with how I've done. I enjoyed it all"

Participants also expressed agreement with the ethicality, coherence, and perceived effectiveness of the study procedures.

"All of the testing has been unobtrusive and seemed very sensible"

"It's very important … it's important for people in the future …. All tests like you do are important … Future generations have still got to get old"

"They're all very worthwhile and very good"

"Perfectly alright … It's all good to have these tests as you don't know yourself"

3.1.4.2.2 Other procedures

Some participants expressed that other aspects of the SPPB, the acceptability of which were not formally assessed in this study, were more burdensome.

"Apart from 'getting up from the chair' [chair stands] it was no effort"

"The only one that really got me was 'the chair' [chair stands]"

"The only thing was the balance thing [tandem stand]"

3.1.4.2.3 Research participation

Although not the primary focus of this study, participants expressed comments relating to their reasons for participating in research. Common themes that emerged were around the desire to help others and feeling that they had been able to provide a service.

"If it helps anyone else to get better then so be it"

"I do them because I know that I'm helping to improve things"

"If it's gonna be useful to you and to someone else that's good enough for me"

"I'm glad to be of service to someone – whatever helps you and your research"

"I'm glad that I was able to help …"

Other participants expressed that they felt they had been able to learn things through participating in research, which had benefitted them personally.

"I just find I learn something and you learn something. My motivation is I want to see the boundaries pushed back"

"We found out how these things work"

"I enjoyed it all – interesting and educational"

"I've just been really interested in what you've done"

Providing the option of being able to have follow-up conducted in the participants' own homes was also considered very positively.

"Grateful to visitors - we enjoyed"

"I've enjoyed you coming and seeing you … it gives you an insight into what's going on"

"I'm pleased that you're able to come to me and I've not got to travel anywhere …"

3.1.5 Discussion

This is the first study to formally evaluate the acceptability of handgrip strength, gait speed, quadriceps ultrasonography, and BIA in older adults with or without frailty during and following hospitalisation. Overall, our results showed that all tests were very acceptable to participants. Our muscle quantity/quality assessments (ultrasonography and BIA) were at least as acceptable as muscle function assessments (handgrip strength and gait speed). If anything, there was a suggestion of increased perceived burden with muscle function assessments, which relates to these tests requiring the participant to actively initiate the test. Importantly, no difference in acceptability was demonstrated with frailty. This is important, as these tests are often used to evaluate frailty, and it is important that there is not a bias against participation of frail older adults in testing. However, as acceptability scores were very positive overall for all groups and all tests, the margin of any difference would be very small.

Coherence was scored high across all outcomes; this was concordant with qualitative responses, with a recurrent emerging theme that participants considered these measures to be important. Interestingly, there were no obvious variations in opportunity costs between tests, which relates to the participants' perceptions of how time-consuming the tests were. Handgrip strength, gait speed, and BIA are certainly quicker to administer than ultrasound. However, there was no suggestion that participants considered any tests any more time-consuming than others; participants considered the time taken to complete each assessment acceptable. Acceptability of aspects of the SPPB other than gait speed were not formally examined as part of this study. However, there was a suggestion from our qualitative results that the other parts of the SPPB (balance and chair stands) may be considered more burdensome to participants. This is important as this may affect compliance with these aspects of the tests i.e. if participants recall that these parts of the test were burdensome on previous testing they may be less likely to agree to repeat them on subsequent testing. Nonetheless, we consider these results vitally important in demonstrating that all tests were at least as acceptable as each other. We consider these to be valuable results towards integration of these measures into clinical practice, and in development of future clinical trials and studies. The results of our studies may also serve as standards when assessing acceptability of other tests in similar populations e.g. muscle biopsies.

Although the purpose of this study was to determine acceptability, our qualitative results considering research participation are of relevance towards planning future clinical trials/studies in older people. Drivers for participation in research were altruism (wanting to help patients in the future), feeling that they were "giving back" towards the hospital (being

of service), and the opportunity to learn/develop their own knowledge. These reasons are consistent with motivators that have been demonstrated elsewhere (McCann et al., 2011; Mein et al., 2012). The option for the study to be performed in participants own homes was reviewed positively. Where practical, this should be considered within study protocols involving older adults. Further research evaluating reasons why patients don't take part in research would be of further value in ensuring that research participation is representative of the patient population.

3.1.5.1 Study strengths

This is the first study to specifically evaluate the acceptability of the measures described to patients during and post- hospitalisation. The questionnaire devised for this study was multi-faceted and developed from recognised domains within acceptability (Sekhon et al., 2017). The simplicity of the survey ensured high completion rates, enabling gathering of both quantitative and qualitative results. Additionally, obtaining feedback at the end of study completion enabled participants to have appropriate time to really consider their feedback on participation, and to be able to provide this in a comfortable environment (either their own home or a quiet clinic room). At this stage participants had also completed the assessments multiple times so were familiar with the tests. This ensured higher completion/response rates.

3.1.5.2 Study limitations

We acknowledge that there are a number of limitations to this study. There is no agreed standard way of assessing acceptability of a medical test. Firstly, the questionnaire itself was devised by the study team. It is unknown how these results would compare against other tests that are commonly used in clinical practice i.e. we do not know whether these results represent "above average" acceptability. Additionally, perceived acceptability of tests may be biased by the agreement of participants to participate in the study in the first place and to complete follow-up; patients who refused to participate and those who did not complete follow-up may have responded differently. Unfortunately, this is an inevitable bias of any study that aims to assess acceptability via participant responses; it would not be possible to assess acceptability of a study procedure in a participant who had not agreed to participate. Results may also be biased by the fact that the questionnaires were administered by the same researcher who conducted the muscle quantity and function assessments; participants may have wished to ingratiate themselves with the research team (Ko et al., 2009). We also acknowledge that religious or cultural differences may affect the results of this study. The majority of participants were White British or Irish and we did not collect personal information about religious beliefs. Acceptability of tests may be viewed differently in other groups e.g. individuals of some religious backgrounds may consider quadriceps ultrasonography to be more personally obtrusive (Karyono et al, 2017). As described, feedback was obtained after the participants' final follow-up assessments, although we consider this a strength, this can also be considered a limitation. Participants may have responded differently if they had been asked to complete feedback in hospital. It is important

to consider that some participants had cognitive impairment and were unable to recall the initial tests, which did not obviously affect responses.

3.1.6 Conclusions

The results of this study may serve as standards for future acceptability studies e.g. when evaluating the acceptability of muscle biopsies. Handgrip strength, gait speed, BIA, and US quadriceps are acceptable to tests to older adults when performed during and after hospitalisation. This applies to those with and without frailty. We recommend the integration of these tests into clinical practice and future research, where these are considered of clinical utility.

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Chapter 3.2 – The feasibility of conducting acute sarcopenia research in hospitalised older patients: a prospective cohort study

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Authors: **Welch C**, Greig C, Majid Z, Masud T, Moorey H, Pinkney T & Jackson TA

Author contributions: CW designed the research question and study protocol. TAJ, CAG, and TM all provided supervision to CW and contributed towards design of the study protocol. TP contributed towards the study protocol. ZM and HM assisted with data collection. CW led collection of research data and analysed the results. All authors read and agreed the final submitted manuscript.

3.2 The feasibility of conducting acute sarcopenia research in hospitalised older patients: a prospective cohort study

3.2.1 Abstract

Purpose: To assess feasibility of conducting acute sarcopenia research in complex populations of hospitalised older adults.

Methods: Patients ≥70 years-old were recruited to three cohorts: elective colorectal surgery, emergency (abdominal) surgery, medical patients with infections. Participants were recruited to the elective cohort in preoperative assessment clinic, and acutely admitted participants from surgical and medical wards at the Queen Elizabeth Hospital Birmingham. Serial measures of muscle quantity (ultrasound quadriceps, bioelectrical impedance analysis), muscle function (hand grip strength, physical performance), and questionnaires (mini-nutritional assessment, physical function) were performed at baseline, within 7 (+/-2) days of admission/ surgery, and 13 (+/-1) weeks post admission/surgery. Feasibility outcomes were assessed across timepoints including recruitment and drop-out rates, and procedure completion rates.

Results: Eighty-one participants were recruited (mean age 79, 38.3% female). Recruitment rates were higher in elective (75%, 24/32) compared to emergency surgery (37.2%, 16/43), and medical participants (45.1%, 41/91; p=0.003). Drop-out rates varied from 8.3 – 19.5% at 7 days, and 12.5 – 43.9% at 13 weeks. Age and gender did not differ between patients assessed for eligibility, approached, or recruited. Completion rates were highest for ultrasound quadriceps (98.8%, 80/81 across all groups at baseline). Gait speed completion rates were lower in medical (70.7%, 29/41) compared to elective participants (100%, 24/24) at baseline.

Conclusion: Higher participation refusal and drop-out rates should be expected for research involving recruitment of participants from the acute setting. Assessment of muscle quantity/quality through ultrasound is recommended in early stage trials in the acute setting, where completion rates of physical performance testing are expected to be lower.

3.2.2 Background

Acute sarcopenia is defined by acute reductions in muscle quantity/quality and/or function (strength or physical performance) leading to incident sarcopenia within six months, and normally occurs follows a stressor event (Cruz-Jentoft et al., 2019). It is an increasingly recognised condition in hospitalised patients and older adults are considered particularly vulnerable (Welch et al., 2018). Interventional trials are urgently needed to prevent and treat this condition. However, this is an inherently complex population, and trial design needs to be pragmatic to enable clinical translation into the real world (Welch et al., 2020a). This study presents feasibility data from a prospective observational cohort study of acute sarcopenia, with direct relevance towards trial design for targeted interventions.

3.2.3 Methods

3.2.3.1 Study setting and design

Participants were recruited from the Queen Elizabeth Hospital Birmingham (QEHB) from May 2019 to April 2021. Recruitment was paused between March 2020 – September 2020, and from January 2021 – March 2021 due to the Coronavirus 2019 (COVID-19) pandemic for safety reasons, and to enable redeployment of clinical staff. The full protocol for this study has been published previously (Welch et al., 2020b). We aimed to involve three cohorts of older patients: elective colorectal surgery, emergency abdominal surgery, and medical patients. Elective patients were recruited from preoperative assessment clinic, with measurements taken prior to admission, within 48 hours postoperatively, 7 (+/-2) days postoperatively, and 13 (+/-1) weeks postoperatively. Emergency surgery patients were recruited from surgical wards preoperatively or postoperatively, with measures taken preoperatively (if possible), within 48 hours postoperatively, 7 (+/-2) days postoperatively, and 13 (+/-1) weeks postoperatively. Medical patients were recruited from medical wards within 48 hours of admission, 7 (+/-2) days post-admission, and 13 (+/-1) weeks post-admission. Follow-up at 13 weeks took place in the participant's own home or the Inflammation Research Facility, QEHB. An amendment was added during the COVID-19 pandemic to enable telephone follow-ups at 13 weeks.

3.2.3.2 Participant population

All participants were aged 70 years and older and provided written informed consent, or personal or professional consultee declaration was obtained if they were unable to consent for themselves during hospitalisation. If provided written informed consent, additional (optional) consent was obtained for them to remain in the study in the event that they should be unable to consent for themselves during hospitalisation. The elective cohort included patients expected to undergo major colorectal surgery, the emergency surgery cohort included emergency admitted patients who had undergone or were planned to undergo emergency abdominal surgery, and the medical cohort included emergency admitted patients with acute bacterial infections. Following an amendment, patients with symptomatic COVID-19 were also included within the medical cohort (Welch et al., 2021a). Pre-specified exclusion criteria for all cohorts were inability to understand verbal English, inability to mobilise prior to admission, or life expectancy less than 30 days. Participants were identified by clinicians who were embedded within the direct care clinical team.

3.2.3.3 Procedures

3.2.3.3.1 Ultrasound quadriceps

At each visit, rectus Femoris (RF) and Vastus Intermedius (VI) were imaged using B-mode ultrasonography (Venue 50, GE Healthcare) bilaterally and thickness measurements taken not including the fascia, as described previously. Bilateral Anterior Thigh Thickness (BATT) was

calculated as the total thickness of all four muscles (right RF + right VI + left RF + left VI) (Wilson et al., 2019).

3.2.3.3.2 Bioelectrical Impedance Analysis

Bioelectrical Impedance Analysis (BIA) was performed using the Bodystat Quadscan 4000 at each visit. Cardiac devices were considered contraindications to this. Weight and height were used to estimate skeletal muscle mass from resistance and reactance, using previously validated equations (Welch et al., 2020b).

3.2.3.3.3 Handgrip strength

Handgrip strength was measured using a Jamar hydraulic dynamometer by asking the participants to "squeeze as hard as [they] can". This was measured with the participant sat out with the elbow bent at 90^{o} where possible (Roberts et al., 2011). Handgrip strength was measured in the bed where participants were unable to sit out in a chair.

3.2.3.3.4 Physical performance

Either usual gait speed alone (four metre course) or Short Physical Performance Battery (SPPB) (Guralnik et al., 1994) were measured at each visit (except for the surgical populations within 48 hours of surgery).

3.2.3.3.5 Questionnaires

Questionnaires were administered at baseline, 7-day, and 13-week visits including Activities of Daily Living (ADLs – Katz (Katz et al., 1963), and Lawton (Lawton and Brody, 1969)), and Patient Reported Outcome Measures Information System (PROMIS® (Tatsuoka et al., 2016)) Physical Function. Mini-Nutritional Assessment (MNA) Full Form (Vellas et al., 1999) was administered at baseline and 13-week follow-up. An acceptability questionnaire was administered at the final visit.

3.2.3.3.6 Other assessments

Frailty was assessed using a Frailty Index (FI) (Rockwood and Mitnitski, 2007), Clinical Frailty Scale (CFS) (Rockwood et al., 2005), and Fried phenotype definition (Fried et al., 2001), as detailed in the original protocol (Welch et al., 2020b). Activities of Daily Living (ADLs) were defined by a combined score of Katz (basic) (Katz et al., 1963) and Lawton (instrumental) (Lawton and Brody, 1969) ADLs. Common selected morbidities were categorised as binary variables. Delirium was assessed for by the geriatrician researcher and defined according to the Diagnostic and Statistical Manual of Mental Disorders 5 (American Psychiatric Association, 2013). Source of infection in the medical cohort, and surgical approach in the surgical cohorts were extracted from routinely collected clinical information. Laparoscopic approach includes extended laparoscopic and assisted laparoscopic approaches. Open approach includes laparoscopic surgery converted to open intra-operatively. Other procedures/assessments performed as part of the study included step count using Fitbit Inspire devices (optional), and

venepuncture (optional) within 48 hours of surgery or admission, and prior to admission in the elective cohort.

3.2.3.4 Feasibility outcomes

We recorded numbers of patients who were identified, approached, and recruited for each cohort, and reasons for non-participation. Age and gender were extracted from routinely collected clinical information for patients who were assessed for eligibility and approached to participate but not recruited to the clinical study. Drop-outs and reasons were recorded at each stage. Where it was not possible to perform specific assessments at each visit, this was also recorded. In the case of physical performance testing, if the participant was able to attempt the test but physically unable to complete it, this was considered as completed. However, if the participant declined testing, or it was unsafe or impractical to do so, then this was considered not completed.

3.2.3.5 Statistical analysis

The study was originally powered to assess within group differences in PROMIS scores (minimally clinically important difference of 6) from baseline to 13 week follow-up (56 participants in each cohort; 45 to follow-up with 25% drop-out rate) (Welch et al., 2020b). Due to the study being paused during the COVID-19 pandemic, the recruitment target was revised to enable assessment of differences in PROMIS scores across groups (i.e. minimum of

45 to follow-up across groups). The analysis presented in this manuscript presents the overall feasibility results; a further power calculation was not derived for this analysis. Statistical analysis was performed using IBM SPSS Statistics 26. Baseline characteristics are summarised as means (SD), and frequencies. One-way analysis of variance (ANOVA), Kruskal-Wallis, and chi-squared tests were used to assess for significance of differences in characteristics between each cohort, and between patients assessed for eligibility, approached, and recruited to the study. Cochran's Q test was used to assess for significance of drop-out rates within groups. Linear mixed models were used to assess for significance of differences in age and FIs within groups. Chi-squared tests were used to assess for significance of drop-out rates between groups, and gender and cognitive disorder differences within and between groups. One-way ANOVA tests were used to assess for significance of differences in age and FIs between groups. One-way ANOVA tests were used to assess for significance of differences in days to follow-up between groups, and chi-squared tests were used to assess for significance of differences in rates of individual assessment completion between groups.

3.2.4 Results

3.2.4.1 Participant characteristics

Table 3.2-1 shows the characteristics for all participants across the three cohorts. Eighty-one participants were recruited across all cohorts (24 elective surgery, 16 emergency surgery, 41 medical). The mean age of all participants was 79 years-old, and 38.3% (31/81) were female. The majority of participants (93.8%, 75/80) were White British. Mean Body Mass Index (BMI)

was 26.7, with no significant difference across cohorts. Participants recruited to the medical cohort were older, with greater risk of being malnourished, higher FIs, higher CFS, lower ADL scores, and greater rates of ischaemic heart disease than the surgical cohorts. There were greater rates of cancer at baseline in the elective cohort, relating to the indication for surgery. The most common source of infection within the medical cohort was respiratory. The majority of operations (85.7%, 12/14) performed within the emergency surgery group were undertaken through an open approach (i.e. emergency laparotomies), which was significantly higher than the emergency surgery group (34.8%, 8/24; p=0.003).

Table 3.2-1 – Baseline characteristics and outcomes of participants.

		Overall (N=80)	Elective surgery (N=24)	Emergency surgery (N=15)	Medical (N=41)	p value
Baseline characteristics						
Age – mean (SD)		79.2 (6.6)	76.4 (5.3)	75.5 (4.2)	82.1 (6.7)	<0.001[a]
Gender – Females % (N)		38.8 (31)	50.0 (12)	33.3 (5)	34.1 (14)	0.400[b]
Ethnicity – % (N)	White British	93.8 (75)	95.8 (23)	100 (15)	90.2 (37)	0.727[b]
	White Irish	2.5 (2)	0 (0)	0 (0)	4.9 (2)	
	Indian	2.5 (2)	4.2 (1)	0 (0)	2.4 (1)	
	Arab	1.3 (1)	0 (0)	0 (0)	2.4 (1)	
Body Mass Index (kg/m^2) – mean (SD)		26.7 (6.5)	26.4 (4.3)	25.0 (5.0)	27.4 (8.0)	0.472[a]
Nutritional status – % (N)	Normal	42.5 (34)	75.0 (18)	40.0 (6)	24.4 (10)	0.001[b]
	At risk	50.0 (40)	25.0 (6)	60.0 (9)	61.0 (25)	
	Malnourished	7.5 (6)	0 (0)	0 (0)	14.6 (6)	
Frailty index – mean (SD)		0.27 (0.11)	0.20 (0.09)	0.25 (0.13)	0.32 (0.09)	<0.001[a]
Clinical Frailty Scale – median (IQR)		4 (3 – 5)	3 (3 – 4)	3 (3 – 4)	5 (4 – 5)	<0.001[c]

Katz and Lawton Activities of Daily Living – median (IQR)		13 (11 – 14)	14 (13 – 14)	13 (10 – 14)	12 (10 – 13)	0.001[c]
Delirium – % (N)		15.0 (12)	8.3 (2)	13.3 (2)	19.5 (8)	0.467[b]
Morbidities – % (N)	Diabetes Mellitus	22.5 (18)	12.5 (3)	26.7 (4)	26.8 (11)	0.374[b]
	Heart failure	5.0 (4)	0 (0)	0 (0)	9.8 (4)	0.135[b]
	Ischaemic Heart Disease	16.3 (12)	0 (0)	20.0 (3)	24.4 (10)	0.033[b]
	Stroke	5.0 (4)	0 (0)	0 (0)	9.8 (4)	0.135[b]
	Cancer	40.0 (32)	91.7 (22)	33.3 (5)	12.2 (5)	<0.001[b]
	Asthma	12.7 (10)	12.5 (3)	13.3 (2)	12.5 (5)	0.996[b]
	Chronic Obstructive Pulmonary Disease	20.0 (16)	20.8 (5)	6.7 (1)	24.4 (10)	0.338[b]
	Anxiety/ Depression	10.0 (8)	12.2 (5)	6.7 (1)	12.2 (5)	0.787[b]
	Pre-existent cognitive impairment	2.5 (2)	0 (0)	0 (0)	4.9 (2)	0.377[b]
Infection source (medical participants only) – % (N)	Respiratory	NA			56.1 (23)	NA
	Urinary				9 (22.0)	
	Skin				7.3 (3)	
	Biliary				2.4 (1)	
	COVID-19				7.3 (3)	
	Unknown origin				4.9 (2)	
Surgical approach – % (N)	Laparoscopic	45.9 (17)	65.2 (15)	14.3 (2)	NA	0.003[b]
	Open	54.1 (20)	34.8 (8)	85.7 (12)		
Outcomes						
Length of stay – median (IQR)		8.5 (5 – 15)	8 (4 – 15)	13 (7 – 20)	8 (5 – 16.5)	0.177[c]
Length of stay <5 days – % (N)		20.5 (16)	34.8 (8)	6.7 (1)	17.5 (7)	
Inpatient death – % (N)		7.5 (6)	8.3 (2)	0 (0)	9.8 (4)	0.463[b]

[a]One-way ANOVA; [b]Chi-squared test; [c]Kruskal-Wallis test

3.2.4.2 Screening and recruitment

Figure 3.2-1 shows the recruitment flowcharts for each cohort. Table 3.2-2 shows patient/participant demographics for participants screened, approached, recruited, and during follow-up. More participants were identified as potentially eligible in the medical cohort compared to the surgical cohorts. However, percentage of patients assessed for eligibility that were approached was lowest in the medical cohort (27.2%, 91/335 vs 71.1%, 32/45 in elective cohort). The most common reasons for non-inclusion in the medical cohort were the inability to mobilise four metres at baseline, or expected discharge the same day. Although expected length of stay did not form part of the prespecified inclusion/criteria, it was generally considered impractical to recruit patients who were expected to be discharged the same day. The percentage of patients who were approached to participate who were recruited was highest in the elective surgery cohort (75%, 24/32) and lowest in the emergency surgery cohort (37.2%, 16/43; p=0.003). In the emergency surgery cohort, the majority of participants (81.3%, 13/16) were recruited post-operatively. There were no significant differences in age or gender within cohorts between patients assessed for eligibility, approached to participate, and recruited. The only significant difference for the group overall was a higher mean age in patients assessed for eligibility, accounted for by the higher weighting of medical patients within this.

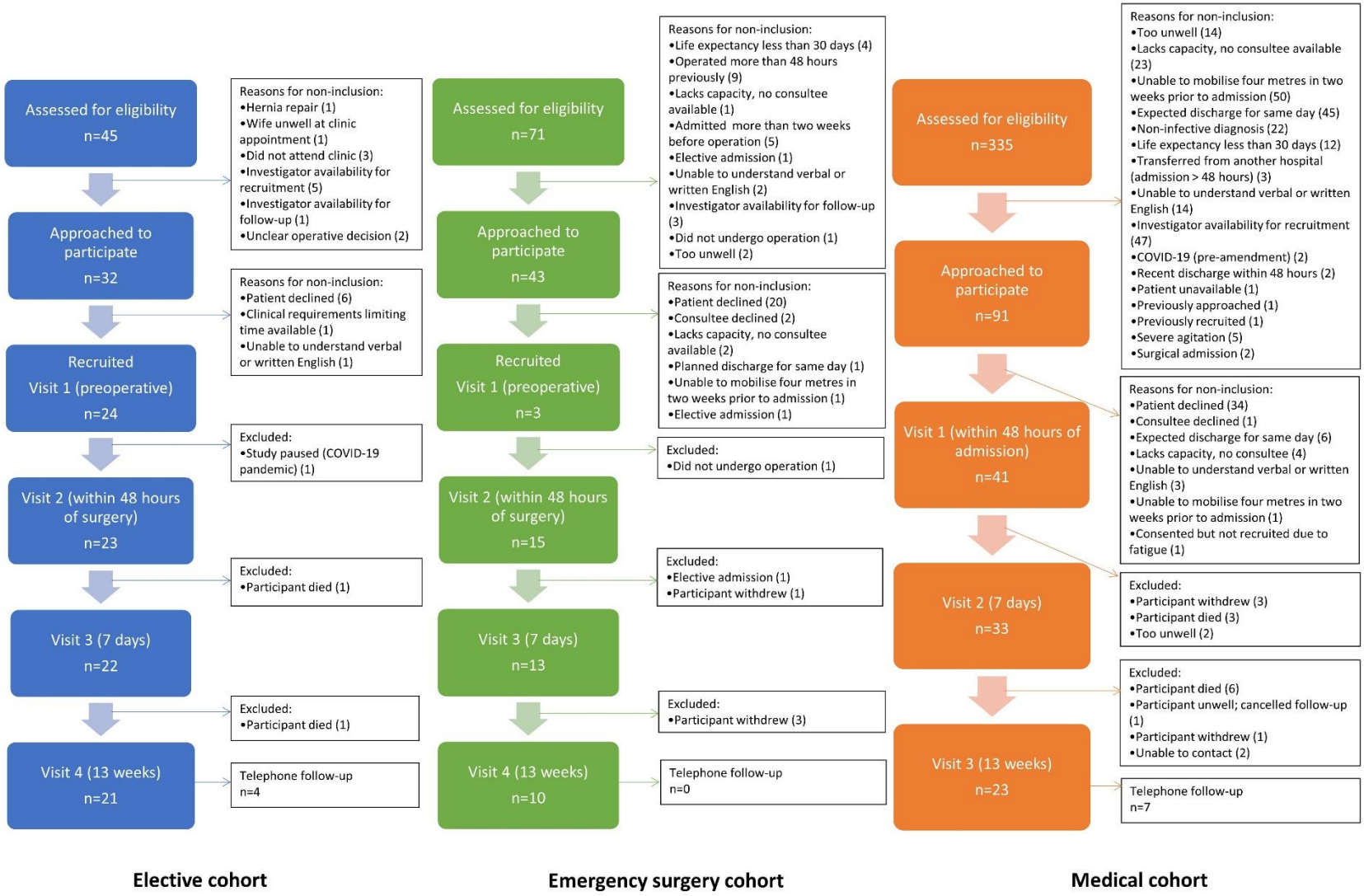

Figure 3.2-1 – Screening, recruitment, and follow-up rates for participants in all cohorts and reasons for non-participation.

Table 3.2-2 – Screening, recruitment, and follow-up rates for participants separated by cohort and characteristics

		Overall	Elective surgery	Emergency surgery	Medical	p value (across groups)
N numbers	Screened – N	451	45	71	335	
	Approached – N (% of screened)	166 (26.8%)	32 (71.1%)	43 (60.6%)	91 (27.2%)	<0.001[a]
	Recruited – N (% of approached)	81 (48.8%)	24 (75%)	16 (37.2%)	41 (45.1%)	0.003[a]
	7 day follow-up – N (% of recruited)	67 (82.7%)	22 (91.7%)	13 (81.3%)	33 (80.5%)	0.470[a]
	13 week follow-up – N (% of recruited)	54 (66.7%)	21 (87.5%)	10 (62.5%)	23 (56.1%)	0.021[a]
	p value (within group)	<0.001[b]	<0.001[b]	<0.001[b]	<0.001[b]	
Age – mean (SD)	Screened	81.2 (7.3)	76.4 (4.8)	76.9 (4.7)	82.8 (7.3)	<0.001[c]
	Approached	79.5 (6.2)	76.7 (5.0)	77.4 (4.6)	81.5 (6.6)	<0.001[c]
	Recruited	79.2 (6.6)	76.4 (5.3)	75.5 (4.2)	82.1 (6.7)	<0.001[c]
	7 day follow-up	78.8 (6.4)	76.1 (4.8)	75.5 (4.2)	81.7 (6.8)	<0.001[c]
	13 week follow-up	78.4 (6.9)	76.0 (4.9)	75.6 (4.2)	82.1 (7.9)	0.004[c]
	p value (within group)	0.001[d]	0.092[d]	0.425[d]	0.598[d]	
Gender – Females % (N)	Screened	50.2 (226)	53.3 (24)	49.3 (35)	50.0 (167)	0.902[a]
	Approached	48.8 (81)	59.4 (19)	44.2 (19)	47.3 (43)	0.389[a]
	Recruited	38.8 (31)	50.0 (12)	33.3 (5)	34.1 (14)	0.400[a]
	7 day follow-up	40.6 (28)	54.5 (12)	38.5 (5)	33.3 (11)	0.227[a]
	13 week follow-up	44.4 (24)	52.4 (11)	40.0 (4)	39.1 (9)	0.701[a]
	p value (within group)	0.228[a]	0.968[a]	0.722[a]	0.127[a]	
Baseline Frailty Index – mean (SD)	Recruited	0.27 (0.11)	0.20 (0.09)	0.25 (0.13)	0.32 (0.09)	<0.001[c]
	7 day follow-up	0.27 (0.11)	0.20 (0.08)	0.25 (0.14)	0.33 (0.08)	<0.001[c]

	13 week follow-up	0.27 (0.11)	0.21 (0.08)	0.23 (0.15)	0.32 (0.09)	<0.001[c]
	p value (within group)	0.755[d]	0.989[d]	0.941[d]	0.973[d]	
Cognitive impairment (delirium and pre-existent) – % (N)	Recruited	17.3 (14)	8.3 (2)	12.5 (2)	24.4 (10)	0.218[a]
	7 day follow-up	15.9 (11)	4.5 (1)	15.4 (2)	23.5 (8)	0.166[a]
	13 week follow-up	13.2 (7)	4.8 (1)	20.0 (2)	18.2 (4)	0.336[a]
	p value (within group)	0.817[a]	0.830[a]	0.876[a]	0.845[a]	

[a]Chi-squared test; [b]Cochran's Q test; [c]One-way ANOVA; [d]Linear mixed models

Considering the reasons why patients who were approached declined to participate, one of the most common reasons was that they felt that they just had "too much going on"; this was frequently cited as a reason for all cohorts. Patients in the surgical cohorts also stated that they wanted to "focus on their operation". In both the emergency surgery and medical cohorts, many patients also frequently stated that they felt "too exhausted", "too unwell", or just "didn't feel up to it". One medical patient who was approached expressed quite frankly that they did not want to "be a guinea pig". Another common reason patients expressed for declining to participate was that, despite assurances, they felt in themselves that they were not appropriate to participate in the research study; "too old", "mobility not good enough", "might not be able to complete assessments", "hearing impairment would make it difficult".

3.2.4.3 Drop-outs and loss to follow-up

Follow-up rates were highest in the elective cohort (7-days: 91.7%, 22/24; 13-weeks: 87.5%, 21/24) and lowest in the medical cohort (7-days: 80.5%, 33/41; 13-weeks: 58.5%, 24/41).

These differences were statistically significant at 13-weeks (p=0.032). Participants who chose to withdraw from the study following recruitment cited similar reasons to those who declined initial participation; “too much going on”, didn’t think their data would be “useful to the study”. There were no statistically significant differences in age, gender, baseline FI, or cognitive impairment (both delirium and pre-existent) within groups between patients recruited and included at follow-up. However, there were non-statistically significant lower rates of participants with cognitive impairment at recruitment remaining in the study at follow-up in the medical cohort. More patients died during their inpatient stay in the medical cohort compared to the surgical cohorts, although this also was not statistically significant (Table 3.2-1). There was no significant difference in the median length of stay between cohorts. However, in the elective cohort 34.8% (8/24) had a length of stay of less than five days, compared to 6.7% (1/15) in the emergency surgery cohort, and 17.5% (7/41) in the medical cohort. The mean number of days to follow-up from visit 2 (surgical cohorts)/ visit 1 (medical cohort) was 5.5 (SD 1.2) days for 7-day follow-up and 90.8 (SD 7.6) days for 13-week follow-up, and there were no significant differences between groups.

3.2.4.4 Feasibility of individual procedures

Table 3.2-3 shows the percentage of each assessment completed at each visit for each patient group, accounting for drop-outs and telephone follow-ups. The procedure with the highest completion rates across all visits was ultrasound quadriceps, with only two single occasions when this was not possible in participants who remained in the study. There was one medical participant in whom ultrasound was attempted, but it was not possible to sufficiently

delineate the muscle borders due to reduced penetration of sound waves through overlying adipose tissue, and one surgical participant for whom ultrasound was abandoned post-operatively due to agitation. This included completion in a number of different settings, with participant standardised in the position with the upper body semi-upright, and the knees extended in the natural resting position.

Table 3.2-3 – Completion rates of individual procedures separated by cohort and study visit

		Overall	Elective surgery	Emergency surgery	Medical	p value
Visit 1/ Baseline						
Elective – preoperative assessment clinic Emergency surgery – preoperative (questionnaires may be post-operative) Medical – within 48 hours of admission	Bioelectrical Impedance Analysis	88.2% (60/68)	91.7% (22/24)	100% (3/3)	85.4% (35/41)	0.607
	Ultrasound quadriceps	98.5% (67/68)	100% (24/24)	100% (3/3)	97.6% (40/41)	0.716
	Handgrip strength	100% (68/68)	100% (24/24)	100% (3/3)	100% (41/41)	NA
	Gait speed	81.5% (53/65)	100% (24/24)	NA	70.7% (29/41)	0.003
	Other physical performance tests	83.1% (54/65)	100% (24/24)	NA	73.2% (30/41)	0.005
	PROMIS Physical Function	98.8% (80/81)	100% (24/24)	93.8% (15/16)	100% (41/41)	0.128
	Other questionnaires	98.8% (80/81)	100% (24/24)	93.8% (15/16)	100% (41/41)	0.128
	Venepuncture	75% (51/68)	100% (24/24)	33.3% (1/3)	63.4% (26/41)	0.001
Visit 2 (surgical)						
Elective – within 48 hours of surgery Emergency surgery –	Bioelectrical Impedance Analysis	89.5% (34/38)	87.0% (20/23)	93.3% (14/15)	NA	0.531
	Ultrasound quadriceps	97.4% (37/38)	95.7% (22/23)	100% (15/15)	NA	0.413
	Handgrip strength	89.5% (34/38)	87.0% (20/23)	93.3% (14/15)	NA	0.531

within 48 hours of surgery	Venepuncture	65.8% (25/38)	60.9% (14/23)	73.3% (11/15)	NA	0.429
Visit 3 (surgical)/ Visit 2 (medical)						
Elective – 7 (+/- 2) post-operative Emergency surgery – 7 (+/- 2) days post-operative Medical – 7 (+/-2) days post-admission	Mean (SD) days from visit 2 (surgery)/ visit 1 (medical)	5.5 (1.2)	5.4 (1.0)	5.0 (1.4)	5.8 (1.1)	0.116
	Bioelectrical Impedance Analysis	87.0% (60/69)	86.4% (19/22)	92.3% (12/13)	85.3% (29/34)	0.811
	Ultrasound quadriceps	100% (67/67)	100% (22/22)	100% (32/32)	100% (13/13)	NA
	Handgrip strength	98.5% (66/67)	95.5% (21/22)	100% (13/13)	100% (32/32)	0.354
	Gait speed	88.1% (59/67)	86.4% (19/22)	92.3% (12/13)	87.5% (28/32)	0.864
	Other physical performance tests	84.4% (27/32)	NA	NA	84.4% (27/32)	NA
	PROMIS Physical Function	94.1% (64/68)	90.9% (20/22)	100% (13/13)	93.9% (31/33)	0.542
	Other questionnaires	98.5% (67/68)	95.5% (21/22)	100% (13/13)	100% (33/33)	0.346
	Fitbit data	51.5% (35/68)	54.5% (12/22)	38.5% (5/13)	54.5% (18/33)	0.580
Visit 4 (surgical)/ Visit 3 (medical)						
Elective – 13 (+/-1) weeks post-operative Emergency surgery – 13 (+/-1) weeks post-operative Medical – 13 (+/-1) weeks post-admission	Mean (SD) days from visit 2 (surgery)/ visit 1 (medical)	90.8 (7.6)	89.6 (6.7)	88.7 (2.9)	93.0 (9.3)	0.219
	Bioelectrical Impedance Analysis	90.0% (36/40)	94.1% (16/17)	80.0% (8/10)	92.3% (12/13)	0.470
	Ultrasound quadriceps	100% (40/40)	100% (17/17)	100% (10/10)	100% (13/13)	NA
	Handgrip strength	100% (40/40)	100% (17/17)	100% (10/10)	100% (13/13)	NA
	Gait speed	100% (40/40)	100% (17/17)	100% (10/10)	100% (13/13)	NA

	Other physical performance tests	97.5% (39/40)	100% (17/17)	100% (10/10)	92.3% (12/13)	0.345
	PROMIS Physical Function	98.1% (53/54)	100% (21/21)	100% (10/10)	95.7% (22/23)	0.503
	Other questionnaires	98.1% (53/54)	100% (21/21)	100% (10/10)	95.7% (22/23)	0.532

Completion rates were highest in the elective group at recruitment. A significantly lower proportion of medical participants were able to complete gait speed testing at recruitment compared to elective participants (70.7%, 29/41 vs 100%, 24/24; p=0.003). Completion rates were higher at 13-week follow-up compared to during hospitalisation in all groups, with 100% of ultrasound quadriceps, handgrip strength, and gait speed testing completed in all groups. All elective participants agreed to venepuncture at baseline assessment, compared to 63.4% (26/41) of medical participants (p=0.001). However, rates were lower post-operatively at 60.9% (14/23) in elective participants. Fitbit data during hospitalisation was collected for 51.5% (35/68) of participants across all groups, with no significant difference between groups.

3.2.4.5 Capacity, delirium, and cognitive impairment

Consultee declaration was obtained at recruitment in 10% (4/41) of medical participants and 12.5% (2/16) of emergency surgery participants, who were considered to lack capacity at time of recruitment. Consultee declaration was also obtained for an additional medical participant who demonstrated ongoing loss of capacity during the study after initially providing informed consent to participate. Across all cohorts, 97.4% (74/76) of participants provided additional consent to remain in the study in the event that they should be unable to make decisions for

themselves during the course of the study. The overall prevalence of delirium in all participants at any point in the study was 15.0% (12/80). This was lowest in the elective cohort (8.3%, 2/24), and highest in the medical cohort (19.5%, 8/41). No participants in the surgical cohorts had pre-existent cognitive impairment. The prevalence of pre-existent cognitive impairment within the medical cohort was 4.9% (2/41).

3.2.5 Discussion

This study provides important feasibility data on conducting acute sarcopenia research in a complex real-world patient population. The recruitment and drop-out rates demonstrated in this study should be used to guide recruitment targets for future cohort studies and interventional trials. Participation refusal and withdrawal rates were lowest in the elective surgery cohort. This is likely to relate to the recruitment environment within the outpatient department, and the patient's own clinical stability. However, this cohort was also younger and less frail than the medical cohort. This may also have impacted upon participation rates, although there was no evidence that patients were more likely to drop-out from the study if they were older or more frail.

The reasons that patients and participants expressed for refusal to participate or withdrawal from the study are illuminating. Previous studies have recurrently shown that older adults are under-represented in clinical trials (Thake and Lowry, 2017). However, despite reassurances, many patients expressed that they felt they were "too old" for research. In our previous study,

we demonstrated that key drivers for research participation amongst older adults were the ability to “give back”, and being able to learn something different (Welch et al., 2021b). Medical professionals should strive to encourage active participation of older adults in research by engaging with them, and demonstrating how their participation could help other people in the future. Recruitment of participants when clinically stable, and ideally in an outpatient or community setting is encouraged where possible. However, for studies evaluating the acute effects of hospitalisation, this is often not practical. A simplified consent process may assist when patients are especially exhausted from their illness.

Delirium and dementia are common in older adults, and mental capacity may fluctuate throughout the course of hospitalisation (Geriatric Medicine Research Collaborative, 2019, Jackson et al., 2016). We have shown significant results that, in participants who are able to provide informed consent at recruitment, nearly all would be happy to remain in the study in the event that they were to lose capacity during the course of the study. We consider that all studies involving hospitalised older patients should include this specific consent. In participants who exhibit ongoing loss of capacity during the course of the study, a personal or professional consultee may be consulted in line with the participant’s wishes and national legislation. The overall rates of delirium in this study were similar to the prevalence demonstrated in previous studies in medical and surgical patients (Geriatric Medicine Research Collaborative, 2019, Geriatric Medicine Research Collaborative, 2021). However, the rates of pre-existent cognitive impairment were lower than demonstrated in previous studies (Geriatric Medicine Research Collaborative, 2019). Although this likely partially relates to higher rates of functional impairment (i.e. inability to walk four metres at baseline) in

patients with advanced dementia, and higher rates of non-infective reasons for admission (e.g. falls, social concerns) (Toot et al., 2013), this potentially suggests a bias in recruitment where it was not possible to recruit participants if consultees were unavailable.

The procedure with the overall highest completion rates was ultrasound quadriceps. Ultrasound provides non-invasive real-time assessment of muscle quantity and quality. We previously showed that ultrasound was highly acceptable to participants, associated with low perceived burden when compared to handgrip strength and gait speed testing (Welch et al., 2021b). Importantly, it was possible to standardise the position that this was performed in in a multitude of settings (outpatient department, inpatient ward, participants' own homes). Previous research with healthy volunteers has demonstrated that BATT will be affected by concurrent hip and knee flexion, but that small variations in tilt of the upper body can be tolerated so long as the knees are kept in natural extension (Welch et al., 2021c). However, ultrasound does require more training and expertise than BIA.

Lower completion rates for BIA are entirely accounted for by participants with cardiac devices in-situ. Recently, BIA has been shown to be potentially safe to be performed in participants with cardiac devices, although it is unclear how the presence of cardiac devices may affect the interpretation of the results (Garlini et al., 2020). It is also important to note that there was one participant in whom it was not technically possible to obtain valid measurements with ultrasound. Gold standard techniques recommended for assessment of muscle quantity are Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) (Cruz-Jentoft et al.,

2019). However, these techniques are not feasible for serial, real-time, or bedside evaluation. We suggest that trials for acute sarcopenia should incorporate both ultrasound and BIA at present, as complementary assessment techniques of muscle quantity. Where possible, gold standard imaging may be performed when stable prior to hospitalisation and at follow-up in studies that aim to explore mechanisms.

Considering the timing of dynamic assessments, the median length of stay across all cohorts was 8.5 days. Therefore, it should be possible to perform repeated measures for most participants, if the first measure is taken within 48 hours of admission. However, a third of elective participants, and almost a fifth of medical participants had a length of stay of four days or less. Additionally, a significant number of identified medical participants were not recruited as they were expected to be discharged. Where feasible, repeated measures can be performed in participant's own homes if they are discharged prior to their planned assessment date, however, this is likely to be impractical and costly for large-scale clinical trials. This may also limit the effectiveness of interventions when these are only delivered to participants during their inpatient stay.

We consider that ultrasound and BIA provide pragmatic tools in demonstrating mechanistic action of effects in interventional trials. These techniques may also demonstrate minimally clinically important difference that might not be demonstrated in other outcomes in preliminary pilot studies. The incorporation of muscle quantity/quality assessment through ultrasound and BIA provides a cost-effective strategy towards demonstrating efficacy in early

interventional trials. However, diagnosis of sarcopenia requires demonstration of loss of muscle function, and not just quantity/quality (Cruz-Jentoft et al., 2019). Completion rates for handgrip strength were higher than physical performance, but it is also recognised that handgrip strength may be affected by fatigue. Trials of interventions for acute sarcopenia should continue to incorporate assessment of muscle function, but the protocols should prespecify how expected non-completion rates will be accounted for.

It should also be emphasised that patient-reported outcomes should be embedded into any clinical trial design. The PROMIS Physical Function questionnaire is simple to administer and sensitive to change (Yost et al., 2011). It is sufficiently broad to avoid ceiling and floor effects. Completion rates at each visit were excellent. Importantly, this questionnaire could be administered over telephone follow-ups when real time assessment is not possible.

Venepuncture and Fitbit use were listed as optional aspects of this study. This may explain why completion rates are lower for these to procedures. It was possible to obtain additional blood samples for all participants recruited to the elective cohort. This relates to the structure of the preoperative assessment clinic, with the research team embedded within this. Blood tests are performed routinely for all patients in preoperative assessment; therefore, it was possible to obtain additional samples at the same needle puncture. However, during hospitalisation, routine clinical bloods were frequently taken at different times, and, therefore, taking additional blood tests for research would have necessitated additional needle puncture. As well as participant refusal, lower rates of Fitbit usage are likely to be

multifactorial. As this was an optional part of the study, the research team may have been less invested in promoting this. Participants were admitted to different locations throughout the hospital, and clinical staff may have been unfamiliar with the devices being used for research. At times devices were lost, particularly between bed moves within hospital.

3.2.5.1 Limitations

We recognise that there are a number of limitations to our study. Firstly, we recognise that recruitment rates and drop-out rates may differ in interventional trials. Interventional trials can both positively and negatively affect recruitment, as the perceived potential benefit may be greater, as well as the perceived potential harm. Nevertheless, we consider that the expected identification, recruitment, and drop-out rates demonstrated in our study should guide sample size calculations and recruitment timeframes. Secondly, our feasibility study itself may be under-powered to demonstrate statistically significant differences in participant characteristics. Previous studies have demonstrated lower recruitment rates amongst females compared to males in early phase clinical studies (Yoon et al., 2014). Although not statistically significant, a lower percentage of participants recruited to the emergency surgery and medical cohorts were female. Protocols should pre-specify how recruitment technique will be adapted to ensure equal gender representation in research. Lastly, the participants recruited to this study were predominantly White British. The exclusion of participants who were unable to understand verbal or written English may have led to bias towards this population.

3.2.6 Conclusion

Acute sarcopenia research represents unique challenges. This includes the challenges of recruiting a heterogeneous vulnerable population, and the challenges of recruiting in a complex clinical environment. Completion rates of physical performance tests should be expected to be lower in hospitalised patients compared to completion rates of tests of muscle quantity and quality. Protocols should be carefully and adapted and designed to optimise recruitment, and reduce drop-outs, ensuring that research is acceptable to older adults. Enhancing options for follow-up assessments to include seeing participants in their own homes, and virtually (telephone or video), will assist to reduce drop-out rates. Research participation rates were highest when participants were recruited in the outpatient setting. Embedding observational studies and trial design into ongoing cohort studies may assist with identifying patients and streamlining recruitment.

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4 Characterising dynamic changes in muscle, sarcopenia, and frailty

Chapter 4.1 – Trajectories of muscle quantity, quality, and function measurements in hospitalised older adults

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4.1 Trajectories of muscle quantity, quality, and function measurements in hospitalised older adults

4.1.1 Abstract

Background: Acute sarcopenia is defined by the development of incident sarcopenia (low muscle quantity/quality and function) within 6 months of a stressor event. However, outcome measures for clinical trials have not been validated. This study aimed to characterize changes in muscle quantity, quality, strength, and physical function during and after hospitalization.

Methods: Patients aged ≥70 years admitted for elective colorectal surgery, emergency abdominal surgery or acute infections were recruited from a single university hospital. Assessments were carried out at baseline, and within 7 ± 2 days and 13 ± 1 weeks postoperatively or post-admission.

Results: A total of 79 participants (mean age 79 years, 39% female) were included. Physical function defined by the Patient-Reported Outcome Measures Information System T-score declined from baseline (42.3, 95% CI 40.2–44.3) to 7 days (36.6, 95% CI 34.5–38.8; P = 0.001), with improvement after 13 weeks (40.5, 95% CI 37.9–43.0). Changes in muscle quantity, quality and function measurements were overall heterogeneous, with few significant changes at the study population level. Change in rectus femoris echogenicity over 13 weeks correlated with changes in handgrip strength (r = 0.53; P < 0.001) and gait speed (r = 0.59; P = 0.003) over the same period.

Conclusions: Patient-Reported Outcome Measures Information System T-score provides a sensitive measure of change in physical function in hospitalized older patients. However,

changes in muscle quantity, quality and function measurements were heterogeneous, and not significant at the study population level. Further research should assess for factors that might be predictive of changes within individuals to enable stratified interventions.

4.1.2 Background

Sarcopenia is defined by low muscle strength with low muscle quantity/quality, with cut-offs two standard deviations below means of young healthy reference populations (Cruz-Jentoft et al., 2019). Additional demonstration of low physical performance defines severe sarcopenia. Acute sarcopenia is a condition of acute muscle insufficiency defined by declines in muscle quantity and/or function leading to incident sarcopenia within 6 months, normally after stressor events (Cruz-Jentoft et al., 2019, Welch et al., 2018). However, relative declines that do not meet sarcopenia cut-offs may also be significant (Welch et al., 2018). Acute sarcopenia is considered to occur commonly in older adults after hospitalization. However, changes in muscle quantity, quality and function have not been fully characterized. Characterization is vital to enable robust trial design and accurate interpretation of effectiveness. Ultrasound and bioelectrical impedance analysis are potential methods for measuring muscle quantity/quality in multiple settings (Wilson et al., 2019, Cruz-Jentoft et al., 2019). The present study aimed to characterize changes in muscle quantity, quality and function in hospitalized older adults, and assess the relationship of changes to patient-reported physical function at 1 week and 3 months post-hospitalization. This was considered important in showing the relationship of change to participants' perceived function at each timepoint.

4.1.3 Methods

4.1.3.1 Study design and setting

This was a single-site study at Queen Elizabeth Hospital Birmingham (QEHB), in Birmingham, the UK. Patients were recruited from May 2019 to April 2021. Recruitment was paused March to September 2020, and January to March 2021 due to the coronavirus disease 2019 (COVID-19) pandemic. The protocol has been published previously (Welch et al., 2020a). The study was prospectively registered (NCT03858192). Patients were recruited to three cohorts: elective colorectal surgery, emergency abdominal surgery and general medical patients with acute bacterial infections. Elective participants were recruited from preoperative assessment clinic, and emergency surgery and medical participants were recruited from medical and surgical wards. Baseline assessments were carried out preoperatively in the elective cohort, within 48 h of surgery in the emergency surgery cohort, and within 48 h of admission in the medical cohort. Assessments were repeated at 7 ± 2 days post-hospitalization/surgery, and at 13 ± 1 weeks post-hospitalization/surgery. Follow up was carried out in participants' own homes or the Inflammation Research Facility, QEHB. Due to the COVID-19 pandemic, amendments were added in March 2020 to enable telephone follow up at 3 months and September 2020 to enable recruitment of patients with COVID-19 to the medical cohort (Welch et al., 2021b).

4.1.3.2 Participants

All participants were aged ≥70 years, and either provided written informed consent to participate, or a personal or professional consultee provided written consultee declaration, where they lacked capacity to do so. Prespecified exclusion criteria were life expectancy <30 days, inability to understand verbal/written English, and inability to mobilize 4 m independently 2 weeks before recruitment.

4.1.3.3 Research procedures

Figure 8.3-1 (Appendix 8.3) shows the timing of each procedure within this study separated by cohort.

4.1.3.3.1 Ultrasound quadriceps

Ultrasound quadriceps was carried out at each visit as previously described (Wilson et al., 2019). Participants were positioned on a hospital bed or couch with knees extended in a natural resting position, a firm wedge placed below knee, and upper body reclined to 45° (Welch et al., 2021c). The same position was established when participants were seen in their own home using recliner chairs, home couches or their own bed. Measurements were taken at the midpoint between the joint line of the knee and greater trochanter on each side. Thickness measurements of subcutaneous (SC) tissue, rectus femoris (RF) and vastus intermedius, not including the fascia, were taken in the transverse plane using B-mode

ultrasonography with a linear probe (Venue 50; GE Healthcare, Chicago, IL. USA). Three (or four if >10% variability) measures were taken on each side, and means of individual readings were used for analysis. Bilateral anterior thigh thickness (BATT) was calculated (right RF + right vastus intermedius + left RF + left vastus intermedius). BATT: SC ratio (BATT-SCR) was calculated as BATT divided by (right SC + left SC). A single image was taken in longitudinal planes on both sides. RF grey scale analysis was carried out using Image J software (National Institutes of Health, Bethesda, MD, USA) to determine echogenicity; a marker of muscle quality.

4.1.3.3.2 Bioelectrical impedance analysis

Bioelectrical impedance analysis was carried out at each visit (Bodystat Quadscan 4000; Bodystat Limited, Douglas, Isle of Man). Participants were positioned as described for ultrasound assessment (Welch et al., 2021c). Electrodes were applied to the right hand and foot. All available measures were extracted from the device. The phase angle was recorded as a marker of muscle quality (Norman et al., 2012). Skeletal muscle mass (SMM) was calculated using two equations: SMM-Sergi and SMM-Janssen (Table 8.3-3, Appendix 8.3). Bioelectrical impedance analysis was not carried out in participants with cardiac devices.

4.1.3.3.3 Handgrip strength

Handgrip strength measurement was carried out at each visit using a Jamar hydraulic dynamometer. Participants were positioned in a chair (if able to sit up) or bed, with their

elbow bent at 90^{o}. Participants were advised to “squeeze as hard as [they] can” (Roberts et al., 2011). Two measures were taken on each side, and the best of all four was used for analysis.

4.1.3.3.4 Physical performance

The Short Physical Performance Battery (SPPB; side-by-side stand, semi-tandem, tandem stand, five chair stands and usual gait speed over 4 m) was measured at all visits in the medical cohort, in preoperative assessment clinic and 13 weeks in the elective cohort, and at 13 weeks in the emergency cohort (Guralnik et al., 1994). Usual gait speed alone was measured at 7 days in the surgical cohorts.

4.1.3.3.5 Patient-Reported Outcomes Measurement Information System (PROMIS®) Physical Function

The Patient-Reported Outcomes Measurements Information System (PROMIS) item bank V2.0 Physical Function Short Form 10b questionnaire was administered at baseline, 7 days, and at 13 weeks (Tatsuoka et al., 2016). In emergency surgery and medical cohorts, participants were asked to answer according to perceived physical function 2 weeks before admission. Raw scores were entered into the HealthMeasures Scoring Service, powered by Assessment Center to derive T-scores.

4.1.3.3.6 Sarcopenia diagnosis

Sarcopenia was defined according to previously defined cut-offs as reduced handgrip strength (<27kg in men, <16kg in women) (Cruz-Jentoft et al., 2019), and reduced BATT (<5.44cm in men, <3.85cm in women) (Wilson et al., 2019) and/or reduced SMMSergi (<20kg in males, <15kg in females) (Cruz-Jentoft et al., 2019). We calculated the prevalence of sarcopenia at baseline and the prevalence of acute sarcopenia at 7 days. We also further calculated the prevalence of participants who experienced negative changes in muscle quantity, strength or performance of ≥10%, but who did not meet criteria for sarcopenia at 7 days.

4.1.3.4 Statistical analysis

Statistical analyses were carried out using IBM spss Statistics 26 (IBM Corporation, Armonk, NY, USA). One-way analysis of variance (anova), χ2-tests, Kruskal–Wallis tests and Mann–Whitney U-tests were used to assess for significance of differences in baseline characteristics, and baseline muscle and physical function measurements between cohorts. The study was originally powered (80% power, alpha 0.05) to assess within-group differences in PROMIS scores from baseline to 13 week follow up (56 participants in each cohort; 45 to follow up with 25% dropout rate). Due to the study being paused, the recruitment target was revised for differences across groups (45 to follow up across groups). To enable comparisons across groups, main analyses were carried out across three visits for all groups, to assess changes to 7 days and 13 weeks compared with baseline. Preoperative assessments were used in the elective cohort, and postoperative assessments were used in the emergency surgery cohort

(i.e. at recruitment for most participants). Linear mixed models (normally distributed variables) and generalized linear mixed models (non-normal distributed variables) were used to assess for the significance of differences in muscle and physical function variables between visits, including an interaction term for visit and group. Mixed models are considered robust to effects of missing values. Estimated marginal means were derived from models. Analyses were separated by sex for variables with sex-specific sarcopenia cut-offs. Secondary analyses for within cohort differences across all visits were carried out using linear mixed models and generalized linear mixed models . Change scores from baseline to 7 days and 13 weeks were calculated for all muscle quantity, quality and physical function measurements. Correlation matrices (Pearson and Spearman) of change scores were generated using GraphPad Prism 9. Multivariate analyses were planned to assess if changes in muscle quantity, quality and function measurements within 7 days were predictive of change in PROMIS score at 13 weeks. However, on evaluation of correlation matrices, multivariate analyses were not indicated.

4.1.4 Results

4.1.4.1 Participant characteristics at baseline

Feasibility analyses including screening, recruitment, and drop-outs have been published separately (Welch et al., 2021a). A total of 81 participants were recruited. One participant was excluded from the emergency surgery cohort (elective admission recruited in error). One further emergency surgery participant was excluded from baseline and main analyses, as only preoperative measurements were carried out (did not undergo surgery). Figure 8.3-1

(Appendix 8.3) shows drop-outs within each cohort. Table 4.1-1 shows baseline characteristics for participants, separated by cohort. Participants in the medical cohort were older (mean age 82.1 vs 76.4 in elective surgery cohort, 75.2 in emergency surgery cohort; P < 0.001), at greater risk of being malnourished and more frail than surgical cohorts. There were no significant differences in muscle quantity or quality between cohorts. However, medical participants had lower physical function at baseline in terms of both physical performance (median 0.33 vs 0.76 m/s in elective surgery; P < 0.001) and PROMIS T-scores (36.8 vs 47.7 in elective surgery; P < 0.001).

Table 4.1-1 – Baseline characteristics, and muscle and physical function assessments for participants separated by patient cohort.

		Overall (N=79)	Elective surgery (N=24)	Emergency surgery (N=14)	Medical (N=41)	p value
Baseline characteristics						
Age – mean (SD)		79.1 (6.6)	76.4 (5.3)	75.2 (4.2)	82.1 (6.7)	<0.001[a]
Gender – Females % (N)		39.2 (31)	50.0 (12)	35.7 (5)	34.1 (14)	0.431[b]
Ethnicity – % (N)	White British	93.7 (74)	95.8 (23)	100 (14)	90.2 (37)	0.742[b]
	White Irish	2.5 (2)	0 (0)	0 (0)	4.9 (2)	
	Indian	2.5 (2)	4.2 (1)	0 (0)	2.4 (1)	
	Arab	1.3 (1)	0 (0)	0 (0)	2.4 (1)	
Body Mass Index (kg/m^2) – mean (SD)		26.5 (6.5)	26.4 (4.3)	24.3 (4.3)	27.4 (8.0)	0.303[a]
Nutritional status – % (N)	Normal	41.8 (33)	75.0 (18)	35.7 (0)	24.4 (10)	0.001[b]
	At risk	50.6 (40)	25.0 (6)	64.3 (9)	61.0 (25)	
	Malnourished	7.6 (6)	0 (0)	0 (0)	14.6 (6)	
Frailty index – mean (SD)		0.27 (0.11)	0.20 (0.09)	0.25 (0.14)	0.32 (0.09)	<0.001[a]
Clinical Frailty Scale – median (IQR)		4 (3 – 5)	3 (3 – 4)	3.5 (2.75 – 4)	5 (4 – 5)	<0.001[c]
Baseline muscle and physical function assessments						
BATT (cm) – mean (SD)	Male	4.49 (1.21)	4.67 (1.07)	4.91 (1.11)	4.24 (1.29)	0.318[a]
	Female	3.69 (1.14)	3.60 (1.15)	3.75 (0.70)	3.73 (1.28)	0.953[a]

BATT-SCR – median (IQR)	Male	3.57 (2.32 – 5.09)	4.16 (2.33 – 5.40)	3.98 (2.77 – 5.87)	3.20 (1.95 – 4.24)	0.293[c]
	Female	1.59 (1.15 – 2.65)	1.36 (1.17 – 3.10)	2.32 (0.95 – 2.73)	1.71 (1.12 – 2.80)	0.948[c]
Echogenicity – mean (SD)	Male	63.3 (13.0)	58.3 (13.9)	65.4 (13.6)	65.8 (11.8)	0.272[a]
	Female	70.0 (13.6)	72.4 (16.5)	63.5 (4.8)	70.2 (13.1)	0.485[a]
SMMJanssen (kg) – median (IQR)	Male	24.7 (21.0 – 28.2)	22.6 (20.9 – 30.6)	25.8 (24.0 – 29.6)	24.7 (18.0 – 27.1)	0.702[c]
	Female	16.9 (15.8 – 20.7)	17.9 (14.7 – 25.6)	16.4 (16.0 – 18.8)	16.7 (15.2 – 20.8)	0.274[c]
SMMSergi (kg) – mean (SD)	Male	21.5 (4.7)	21.4 (4.7)	21.9 (2.5)	21.3 (5.7)	0.946[a]
	Female	16.5 (4.9)	17.6 (5.1)	14.0 (1.8)	16.4 (5.2)	0.471[a]
Phase angle (°) – median (IQR)	Male	4.60 (3.90 – 5.30)	4.70 (4.60 – 5.80)	4.20 (3.65 – 4.65)	4.40 (3.80 – 5.30)	0.124[c]
	Female	4.80 (4.00 – 5.50)	5.30 (4.80 – 5.50)	4.30 (3.93 – 4.75)	4.25 (3.65 – 5.50)	0.072[c]
Handgrip strength (kg) – mean (SD)	Male	23.1 (9.3)	26.6 (10.7)	25.3 (9.1)	20.4 (8.1)	0.123[a]
	Female	14.8 (8.1)	19.1 (7.9)	12.2 (5.4)	12.7 (7.9)	0.074[a]
Gait speed (m/s) – median (IQR)		0.58 (0.19 – 0.76)	0.76 (0.67 – 0.89)	NA	0.33 (0 – 0.55)	<0.001[d]
SPPB – median (IQR)		6.50 (1.00 – 9.00)	9.00 (8.00 – 10.75)	NA	1.50 (0 – 5.00)	<0.001[d]
PROMIS T score – mean (SD)		41.1 (9.5)	47.7 (9.5)	42.3 (10.0) *p=0.145*	36.8 (9.0) *p<0.001*	<0.001[a]

[a]One-way ANOVA; [b]Chi-squared test; [c]Kruskal-Wallis test; [d]Mann-Whitney U test

BATT = Bilateral Anterior Thigh Thickness; BATT-SCR = Bilateral Anterior Thigh Thickness: Subcutaneous tissue Ratio; SMMJanssen = Skeletal Muscle Mass (Janssen equation); SMMSergi = Skeletal Muscle Mass (Sergi equation); SPPB = Short Physical Performance Battery; PROMIS = Patient-Reported Outcomes Measurement Information System

4.1.4.2 Dynamic changes in muscle quantity, quality, and function measurements

Table 4.1-2 shows estimated marginal means and 95% confidence intervals for measurements across each visit across groups. There was a general trend across all measures toward reduction at 7 days compared with baseline. However, most changes were not statistically

significant. PROMIS T-scores significantly declined from baseline to 7 days postoperative/post-admission. However, scores recovered toward baseline at 13 weeks, with a similar pattern seen with gait speed (Figure 4.1-1). Figure 8.3-3 shows the prevalence of acute sarcopenia at 7 days, as well as the percentage of participants who experienced negative changes in muscle quantity, strength or physical performance, but who did not meet criteria for sarcopenia at 7 days. Of those participants who did not meet criteria for sarcopenia at baseline, just 22.2% did not experience negative changes of ≥10% or meet the criteria for acute sarcopenia.

Table 4.1-2 – Estimated marginal means derived from linear mixed models and generalized linear mixed models. 95% confidence intervals are shown in brackets

		Baseline	7(+/-2) days post-admission/ surgery	13(+/-1) weeks post-admission/ surgery	p value		
					Visit	Group	Visit*Group
BATT (cm)	Male	4.61 (4.21 – 5.00)	4.20 (3.82 – 4.59)	4.26 (3.69 – 4.82)	0.310[a]	0.012[a]	0.543[a]
	Female	3.69 (3.22 – 4.16)	3.29 (2.76 – 3.82)	3.71 (3.13 – 4.29)	0.430[a]	0.533[a]	0.827[a]
BATT-SCR	Male	4.02 (3.38 – 4.79)	3.64 (3.03 – 4.37)	3.96 (2.83 – 5.54)	0.714[b]	0.033[b]	0.934[b]
	Female	1.97 (1.58 – 2.46)	1.74 (1.39 – 2.17)	2.03 (1.53 – 2.69)	0.592[b]	0.764[b]	0.939[b]
Echogenicity	Male	63.2 (58.7 – 67.8)	64.1 (59.0 – 69.2)	61.0 (53.6 – 68.4)	0.769[a]	0.969[a]	0.561[a]
	Female	68.9 (63.5 – 74.4)	71.9 (64.6 – 79.1)	67.8 (59.7 – 75.9)	0.707[a]	0.916[a]	0.669[a]
SMMJanssen (kg)	Male	25.0 (22.7 – 27.4)	24.1 (21.5 – 27.0)	20.6 (18.7 – 22.7)	0.013[b]	0.004[b]	0.068[b]
	Female	18.5	17.0	18.3	0.694[b]	0.274[b]	0.023[b]

		(16.1 – 21.3)	(14.6 – 19.9)	(15.3 – 21.8)			
SMMSergi (kg)	Male	21.6 (19.9 – 23.2)	21.0 (19.2 – 22.8)	20.6 (17.9 – 23.2)	0.777[a]	0.590[a]	0.981[a]
	Female	16.0 (13.9 – 18.1)	15.3 (13.1 – 17.5)	15.5 (12.4 – 18.5)	0.878[a]	0.037[a]	0.808[a]
Phase angle (°)	Male	5.87 (4.86 – 7.10)	4.47 (4.19 – 4.77)	5.87 (4.86 – 7.10)	0.026[b]	0.485[b]	0.082[b]
	Female	4.95 (4.23 – 5.79)	4.82 (4.15 – 5.59)	5.32 (4.63 – 6.11)	0.556[b]	0.095[b]	0.369[b]
Handgrip (kg)	Male	24.1 (21.1 – 27.1)	23.1 (19.8 – 26.4)	25.7 (21.0 – 30.5)	0.648[a]	0.022[a]	0.549[a]
	Female	14.7 (11.6 – 17.7)	13.4 (10.4 – 16.3)	16.7 (12.7 – 20.7)	0.384[a]	0.002[a]	0.870[a]
Gait speed (m/s)		0.65 (0.58 – 0.73)	0.50 (0.43 – 0.58)	0.66 (0.58 – 0.75)	0.004[b]	<0.001[b]	0.426[b]
SPPB		6.19 (5.24 – 7.32)	4.25 (3.01 – 5.85)	6.99 (5.97 – 8.19)	0.904[b]	<0.001[b]	0.290[b]
PROMIS T score		42.3 (40.2 – 44.3)	36.6 (34.5 – 38.8)	40.5 (37.9 – 43.0)	0.001[a]	<0.001[a]	0.302[a]

[a]Linear Mixed Models; [b]Generalized Linear Mixed Model

BATT = Bilateral Anterior Thigh Thickness; BATT-SCR = Bilateral Anterior Thigh Thickness: Subcutaneous tissue Ratio; SMMJanssen = Skeletal Muscle Mass (Janssen equation); SMMSergi = Skeletal Muscle Mass (Sergi equation); SPPB = Short Physical Performance Battery; PROMIS = Patient-Reported Outcomes Measurement Information System

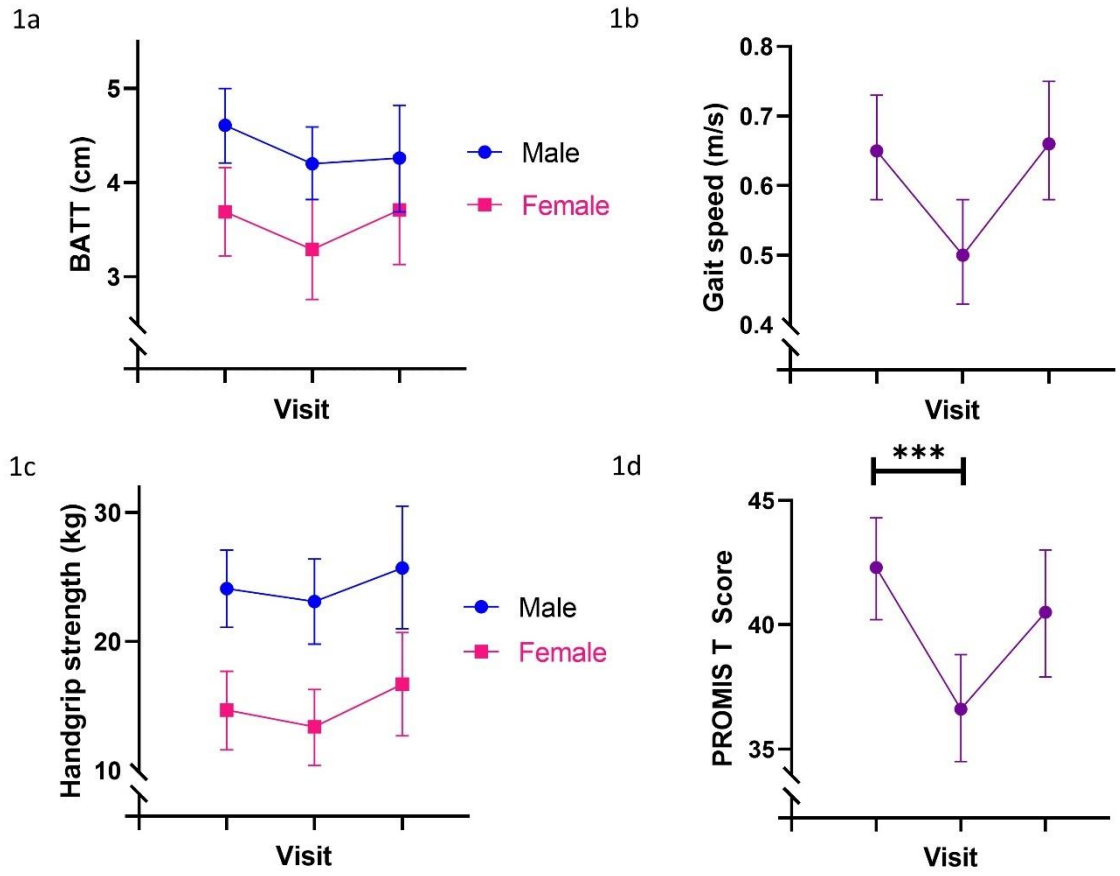

Figure 4.1-1 – Changes in estimated marginal means of muscle quantity and function measurements between visits. Error bars are 95% confidence intervals. BATT=Bilateral Anterior Thigh Thickness

4.1.4.3 Correlations of individual change scores in PROMIS with other measurements

Figure 4.1-2 shows the Pearson correlation matrix for change scores of muscle quantity, quality, and function measurements at 7 days and 13 weeks. Spearman correlations produced similar results (Figure 8.3-2, Appendix 8.3There were no significant correlations with change in PROMIS T-score at 7 days. There were moderate correlations between change in PROMIS T-score at 13 weeks, and changes in PROMIS T-score and SPPB at 7 days, and changes in SMM-Janssen and SPPB at 13 weeks. There were also moderate correlations between the change

in echogenicity at 13 weeks and change in gait speed and SPPB at 7 days, and change in gait speed and handgrip strength at 13 weeks (Figure 3).

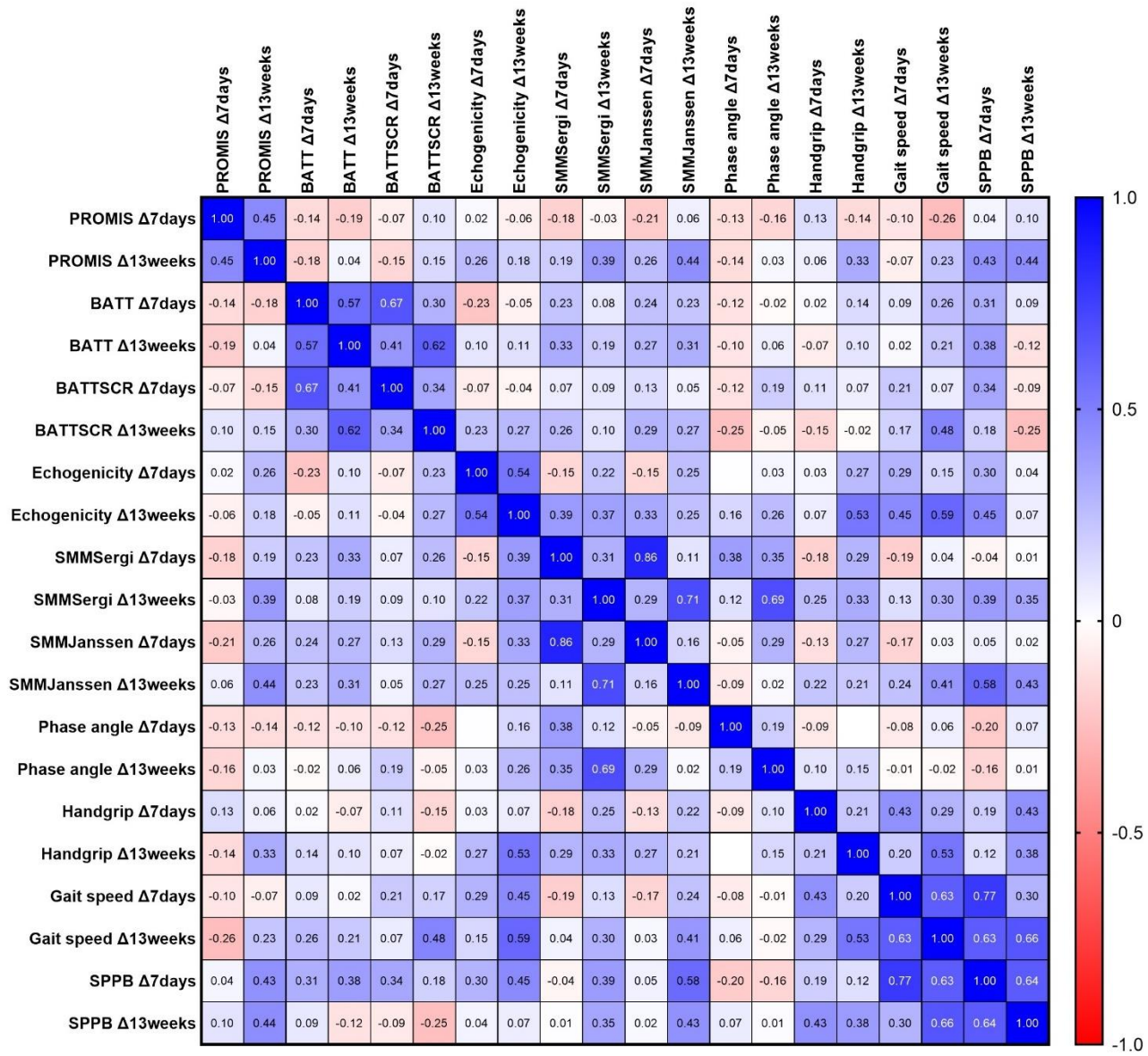

Figure 4.1-2 – Correlation matrix derived from Pearson correlations.

PROMIS=Patient-Reported Outcome Measures Information System, Physical Function; BATT=Bilateral Anterior Thigh Thickness; BATTSCR=Bilateral Anterior Thigh Thickness: Subcutaneous tissue Ratio; SMMSergi=Skeletal Muscle Mass (Sergi equation); SMMJanssen=Skeletal Muscle Mass (Janssen equation); SPPB=Short Physical Performance Battery

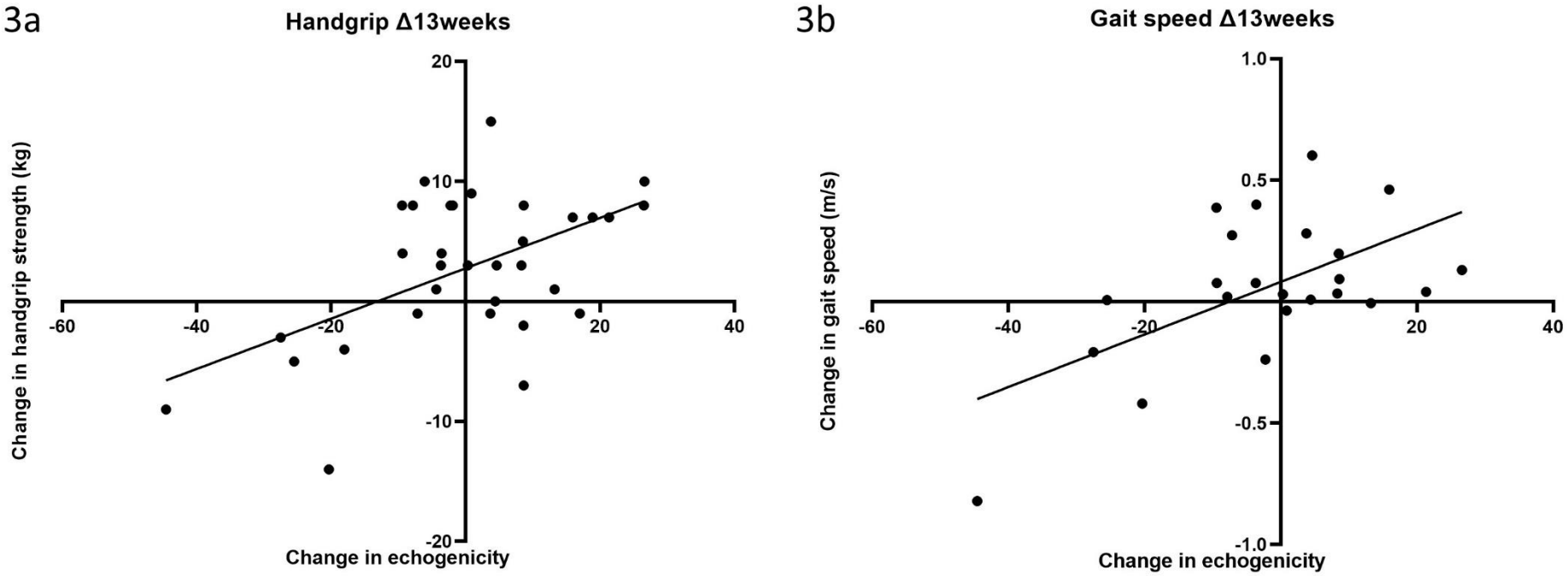

Figure 4.1-3 – Association of echogenicity between handgrip strength and gait speed. Trend lines are derived from simple linear regression.

4.1.5 Discussion

Baseline measurements of muscle quantity and quality did not significantly differ between groups. This is despite medical participants showing greater levels of frailty, being more likely to be malnourished, and having lower patient-reported physical function and physical performance scores. Previous studies have shown lower prevalence of frailty amongst surgical compared to medical patients. However, previous studies evaluating muscle quantity and function in hospitalized older adults have focused on single patient groups (Martone et al., 2017), or analysed changes and differences overall combining different specialty populations (Aarden et al., 2021, Van Ancum et al., 2017b).

Overall, minimal changes in muscle quantity, quality or function were shown at the study population level. This is consistent with previous studies that have not shown significant change in handgrip strength in acutely admitted older adults during hospitalization (Hartley et al., 2020, Van Ancum et al., 2017a), or at 3 months post-hospitalization (Aarden et al., 2021). A previous systematic review demonstrated declines in handgrip strength in electively admitted older adults, but not in acutely admitted patients (Van Ancum et al., 2017a). Conversely, muscle quantity has been shown to decline at 3 months post-hospitalization (Aarden et al., 2021), but not during hospitalization (Van Ancum et al., 2017b), and physical performance has actually been shown to improve in other studies (Aarden et al., 2021, Hartley et al., 2020). This demonstrates complexities in measuring dynamic changes in muscle quantity, quality, and function in heterogeneous populations. It is important to note that whilst changes were not demonstrated at study population level, some individuals

experienced significant negative changes. Previous interventional trials have often examined for effect sizes at study population levels (Welch et al., 2020b). However, unless interventions are targeted towards individuals most likely to experience negative changes, it may not be possible to show effectiveness.

Gait speed declined significantly at 7 days post-admission/postoperatively. However, this might have been affected by factors, such as pain, and restraint from intravenous fluids and catheters. This shows the need for caution when carrying out studies measuring physical performance during hospitalization, where assessment at a single timepoint might provide an incomplete clinical picture. In this hospital, enhanced recovery after surgery, including early mobilization, is part of the standard care for patients undergoing elective colorectal surgery (Rawlinson et al., 2011).

Knee extension strength was not measured as part of this study, but has been shown to decline during hospitalization (Hartley et al., 2020). Knee extension strength has been shown to be more sensitive to change in resistance exercise trials in frail older adults than handgrip strength (Tieland et al., 2015). It is likely that different muscles might respond differently to hospital-associated inactivity/disuse. Hospitalization might be associated with prolonged periods of bedrest, with limited lower limb use, but continuous upper limb use. Lower limb anti-gravity muscles might be more susceptible to declines in function than upper limb muscles.

Where changes did occur, these were infrequently correlated. This suggests there might be multiple mechanisms affecting changes. This is potentially very important to consider, as all changes might be individually significant. Identifying mechanistic pathways for individual changes is imperative to ensure that most suitable outcomes are included within trials that seek to target specific pathways. It is important to consider that many participants experienced negative declines of ≥10% in individual domains, but did not meet the criteria for sarcopenia; some participants experienced declines in all domains without meeting criteria for sarcopenia. This shows the importance of considering dynamic changes, as these relative declines are likely to be individually important.

Notably, changes in PROMIS scores were shown acutely during hospitalization. This confirms that the PROMIS physical function score itself is sensitive to change in an older hospitalized population, and might be an appropriate outcome measure in large-scale clinical studies. However, PROMIS scores are reliant on participants' own perceptions. Although this can be considered a strength, the lack of objectivity means that scores might not be appropriate outcomes for early-stage efficacy trials aimed at showing mechanisms underlying interventions. PROMIS provides a measure of participants' own perceptions of what they are able to do, rather than an objective assessment of what they can do. Responses might, therefore, vary according to mood, cognition, cultural background or outlook on life (Tatsuoka et al., 2016). Responses may also differ when obtained from proxies (Chang et al., 2019).

Changes in PROMIS scores did not clearly correlate with changes in other measurements. This suggests that the PROMIS score may be affected by multidimensional factors, and not just intrinsic muscle factors. Hospitalization might be associated with symptoms of fatigue (van Seben et al., 2020), low mood (Abad et al., 2010), cognitive impairment (van Seben et al., 2020), physical restraints from indwelling catheters and lines (Lee and Malatt, 2011), as well as disease-specific symptoms such as nausea (Singh et al., 2016), pain (van Seben et al., 2020), and breathlessness (Hutchinson et al., 2017). All of these factors might lead to impairments in physical function that are not intrinsically muscle-related. Understanding these factors is imperative to considering how interventions are targeted to prevent negative changes in physical function.

Change in RF echogenicity but not muscle quantity measures correlated with change in function measures (handgrip strength and gait speed) over 13 weeks. Echogenicity is considered to relate to intramuscular adipose deposition, and provides a measure of muscle quality. This suggests that muscle quality may be more important for maintenance of muscle function than muscle quantity. This is consistent with previous cross-sectional research in stable older adults, which showed that RF echogenicity correlated with handgrip strength and gait speed (Wilson et al., 2019). However, change in echogenicity was not associated with change in function over 7 days. This might relate to effects of fluid shifts on echogenicity; increased edema and extracellular fluid (e.g. postoperatively) might lead to reductions in echogenicity, as water will appear more black on ultrasound imaging (Grimm et al., 2013). Fatigue and compliance with handgrip strength and physical performance assessment in the acute setting might impact on these measures. Alternatively, this might suggest that muscle

quality is more relevant in development of chronic sarcopenia, with development over longer time periods.

Importantly, associations do not necessarily imply causation, or direction or causality. Low muscle quality (low echogenicity) might develop as a consequence of reduced muscle activity (presenting as low handgrip strength/gait speed), muscle function might be reduced directly by reduced muscle quality or there might be intermediary factors affecting all measures. Considering trends shown in Figure 3, it should be noted that, although some individuals experienced reductions in muscle quality and function, other individuals experienced improvements. Understanding differences between these groups is imperative toward deciphering mechanisms, and carefully targeting and stratifying interventions.

Large cohort studies to fully characterize changes during and after hospitalization are encouraged, with implementation of techniques, such as latent class association, to understand what is different about those who experience improvements in muscle quantity, quality and function, compared with those who experience declines. Individual follow up to understand how changes impact on much longer-term outcomes would also be beneficial. Such studies could potentially be embedded into longitudinal studies to enable collection of pre-insult measurements, even in unscheduled admissions.

Mechanistic studies are warranted to understand pathways associated with phenotypic changes. Ideally, such studies should incorporate serial muscle biopsies to enable enhanced

understanding that could lead to the development of novel interventions. At the same time, interventional studies should not be delayed, and studies might need to have both applied health and translational remits. Early stage clinical trials might need to pragmatically include multiple outcomes to assess mechanisms and efficacy.

We recognize that there are limitations of the present study. First, due to the need to pause recruitment during the COVID-19 pandemic, this study was underpowered compared with the original planned sample size. The study was powered sufficiently to assess differences across groups, but we cannot rule out the possibility that more significant differences might have been identified within groups in a larger sample size. Second, participants were recruited from a single site, and results might not be broadly representative elsewhere; importantly, most participants were white British. Additionally, recruitment and follow up of participants was led by a single researcher who also carried out the main statistical analysis, and was not blinded to analysis of results. Finally, we acknowledge that effects of missing values and participant dropout are unknown.

Older adults showed acute declines in their own perceived physical function after hospitalization. However, this did not clearly relate to changes in muscle quantity or quality. Changes in muscle echogenicity within 13 weeks of hospitalization were associated with changes in handgrip strength and gait speed. Further research should assess for class associations to enable stratification towards targeted interventions.

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Chapter 4.2 – Induced frailty and acute sarcopenia are overlapping consequences of hospitalisation in older adults

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Author contributions: CW designed the research question and study protocol. TAJ, CAG, and TM all provided supervision to CW and contributed towards design of the study protocol. TP contributed towards the study protocol. ZM and HM assisted with data collection. CW led collection of research data and analysed the results. All authors read and agreed the final submitted manuscript.

4.2 Induced frailty and acute sarcopenia are overlapping consequences of hospitalisation in older adults

4.2.1 Abstract

Objective: To determine the effects of hospitalisation upon frailty and sarcopenia.

Methods: Prospective cohort study at single UK hospital including adults ≥70 years-old admitted for elective colorectal surgery, emergency abdominal surgery, or acute infections. Serial assessments for frailty (Fried, Frailty Index, Clinical Frailty Scale [CFS]), and sarcopenia (handgrip strength, ultrasound quadriceps and/or bioelectrical impedance analysis, and gait speed and/or Short Physical Performance Battery) were conducted at baseline, 7 days post-admission/post-operatively, and 13 weeks post-admission/post-operatively.

Results: Eighty participants were included (mean age 79.2, 38.8% females). Frailty prevalence by all criteria at baseline was higher among medical compared to surgical participants. Median and estimated marginal CFS values and Fried frailty prevalence increased after 7 days, with rates returning towards baseline at 13 weeks. Sarcopenia incidence amongst those who did not have sarcopenia at baseline was 20.0%. However, some participants demonstrated improvements in sarcopenia status, and overall sarcopenia prevalence did not change. There was significant overlap between diagnoses with 37.3% meeting criteria for all four diagnoses at 7 days.

Conclusions: Induced frailty and acute sarcopenia are overlapping conditions affecting older adults during hospitalisation. Rates of frailty returned towards baseline at 13 weeks, suggesting that induced frailty is reversible.

4.2.2 Introduction

Frailty and sarcopenia are known to be related but distinct conditions. The prevalence of both conditions increases with age (Wilson et al., 2017). Frailty is a condition of increased vulnerability and susceptibility to the effects of illness (Clegg et al., 2013). It can be defined phenotypically (Fried frailty) (Fried et al., 2001) or based on the accumulation of increasing numbers of health deficits (Frailty Index) (Rockwood and Mitnitski, 2007). Characteristics included within the phenotypic definition are weakness, slowness, self-reported exhaustion, weight loss, and low physical activity (Fried et al., 2001). Sarcopenia is defined by skeletal muscle insufficiency, with reduced muscle strength being demonstrated with reduced muscle quantity or quality; additional demonstration of low physical performance defines severe sarcopenia (Cruz-Jentoft et al., 2019). Sarcopenia has been shown to overlap especially with Fried frailty (Gingrich et al., 2019). However, previous studies have classically considered the prevalence of frailty and/or sarcopenia at a single timepoint, rather than considering the dynamic nature of these conditions, particularly in the context of acute illness. Induced frailty is an increasingly recognised condition of frailty developing acutely by the effects of illness (Hawkins et al., 2018). Similarly, acute sarcopenia is defined by incident sarcopenia within six months, normally following a stressor event (Cruz-Jentoft et al., 2019, Welch et al., 2018). This study aimed to characterise dynamic changes in frailty and sarcopenia status following hospitalisation in older adults.

4.2.3 Methods

4.2.3.1 Study design and setting

The full protocol for this study has been published previously (Welch et al., 2020). Participants were recruited to this study from the Queen Elizabeth Hospital Birmingham, UK, from May 2019 – April 2021. Recruitment was paused from March 2020 – September 2020 and January 2021 – March 2021 due to the Coronavirus 2019 (COVID-19) pandemic. Three groups of participants were recruited: patients undergoing elective colorectal surgery were recruited from preoperative assessment clinic, patients undergoing emergency abdominal surgery were recruited from general surgery wards, and patients admitted with acute infections were admitted from general medical wards. All participants were aged 70 years and older. Assessments were performed at baseline, 7 (+/-2) days post-admission or post-operatively, and 13 (+/-1) weeks post-admission or post-operatively.

4.2.3.2 Frailty definitions

4.2.3.2.1 Fried frailty phenotype

Fried frailty was defined dichotomously based on the presence of three or more of five characteristics: weight loss, low handgrip strength, low gait speed, self-reported exhaustion, and low physical activity. Low physical activity was defined as per the Frailty Intervention Trial definition (Fairhall et al., 2008), and all other characteristics were defined according to the

original study definition (Table 8.4-1, Appendix 8.4) (Fried et al., 2001). During the COVID-19 pandemic, an amendment was made to conduct telephone follow-ups in place of in person review at 13 weeks. It was, therefore, not possible to assess Fried frailty at this timepoint for these participants.

4.2.3.2.2 Frailty index

The deficits included within the Frailty Index (FI) were adapted from those included within the UK electronic Frailty Index (eFI) (Table 8.4-2, Appendix 8.4) (Clegg et al., 2016). The presence or absence of each deficit was recorded as a binary variable, and the FI was calculated as the number of variables present, divided by the total number measured. The FI was recorded as a continuous variable, with the presence of frailty specifically defined as a score of 0.25 or greater.

4.2.3.2.3 Clinical Frailty Scale

The Clinical Frailty Scale (CFS) (Pulok et al., 2020) was calculated by a single geriatrician based on a Comprehensive Geriatric Assessment, considering activities of daily living, physical and cognitive function, symptomatic burden of morbidities, and perceived vulnerability by the investigator. The CFS was measured on an ordinal scale from 1 – 8 (an additional discrete category of 9 applied to participants who were not otherwise frail but considered to be within the last year of life, but no participants in this study met this criteria) (Figure 8.4-1, Appendix 8.4). The CFS was assessed at baseline by considering the participant's overall function and

health two weeks prior to admission. In contrast, the CFS was calculated at 7 days and 13 weeks, by considering the participant's function and health at that timepoint. At 7 days, some participants were already discharged and assessed at home, others had discharge plans in place, and others required ongoing care and treatment in hospital. The CFS was assessed at this timepoint as a global assessment of health and function involving the patient, and other members of the multidisciplinary team. The stability and trajectory of function and health during hospitalisation were considered when assessing CFS at the timepoint. When considering overall frailty prevalence, a score of 5 or greater was considered consistent with frailty.

4.2.3.3 Sarcopenia definition

Probable sarcopenia was defined by the presence of low handgrip strength alone. Definite sarcopenia was defined by the presence of low handgrip strength with low muscle quantity measured using quadriceps ultrasound (Bilateral Anterior Thigh Thickness [BATT]) (Wilson et al., 2019) and/or bioelectrical impedance analysis (Sergi equation) (Cruz-Jentoft et al., 2019). Severe sarcopenia was defined by additional demonstration of reduced gait speed and/or reduced Short Physical Performance Battery (SPPB) score. Participants were categorised as having sarcopenia with unclear severity if they met criteria for definite sarcopenia but it was not possible to measure physical performance. Cut-offs utilised for diagnosis are available online (Table 8.4-3, Appendix 8.4). When considering overall prevalence, sarcopenia was defined dichotomously according to those with definite sarcopenia and those without. As per

Fried frailty, it was not possible to assess sarcopenia status at 13 weeks in participants where only telephone follow-up was made.

4.2.3.4 Ethical approval

This research was sponsored by the University of Birmingham. Ethical approval was obtained from Wales Research Ethics Committee 4 (19/WA/0036), the Health Research Authority, and the University Hospitals Birmingham NHS Trust Research and Development department. Written informed consent was obtained from all participants who were considered to have capacity to consent for themselves. Written personal or professional consultee declaration was obtained if the participant was considered to lack capacity to consent to participation. The use of both informed consent and consultee declaration was approved by the ethics committee.

4.2.3.5 Statistical analysis

Unless specified otherwise, statistical analyses were performed using IBM SPSS Statistics Version 26 (IBM Corp). All analyses were calculated overall and separated by patient group, to assess for differences across the three timepoints. The original power calculation was derived to identify changes in patient-reported physical function within groups. Unfortunately, it was not possible to recruit to the original target due to the study being paused during the COVID-19 pandemic, and the power calculation was revised to enable analysis of differences across groups rather than within groups. This study represents analysis

of differences in prevalence in frailty and sarcopenia status across timepoints. A post-hoc power calculation specific to this analysis showed that a sample size of 40 participants was able to detect a change in prevalence from 30% to 60% with 80% power and alpha of 0.05. A sample size of 47 participants was able to detect a change in prevalence from 60% to 85% with 80% power and alpha of 0.05.

Estimated marginal means (EMMs) and statistical significance of changes for FIs were calculated from linear mixed models. Statistical significance of change in CFS was calculated considering CFS as an ordinal variable using Skillings-Mack tests using STATA. Skillings-Mack tests are more robust to missing values than Friedman tests, but exclude single measures, with clinical differences across the study population interpreted by median values. To enhance the interpretation of sensitivity to change, EMMs were calculated from Generalized Linear Mixed Methods, considering CFS as a non-parametric continuous variable. Statistical significance of changes in prevalence across the five categories of sarcopenia status were calculated using Skillings-Mack tests. Where ties existed, p values were obtained from a simulated conditional null distribution of Skillings-Mack. Statistical significance of differences in frailty and sarcopenia statuses defined dichotomously between both groups and timepoints were assessed using Chi-squared tests. Change scores between FI, CFS, and sarcopenia categories were calculated for the study population overall. Changes between unclear severity and confirmed or severe sarcopenia were considered as no change. The association of differences in changes between FI, CFS, and sarcopenia were assessed using Spearman's rank correlations. Statistical significance of all analyses was set at $p<0.05$.

4.2.4 Results

Eighty-one participants were recruited to this study. One participant was excluded from analysis as they were recruited in error (elective admission within emergency surgery cohort). Screening, recruitment, and drop-out rates have been published previously (Welch et al., 2021). The recruitment flowchart for this analysis is included in the appendix (Figure 8.4-2, Appendix 8.4). The mean age of participants was 79.2 (6.6) years; 38.8% (31) were female. Baseline characteristics of participants are shown in Table 4.2-1.

Table 4.2-1 – Baseline characteristics of participants

		Overall (N=80)	Elective surgery (N=24)	Emergency surgery (N=15)	Medical (N=41)	p value
Age – mean (SD)		79.2 (6.6)	76.4 (5.3)	75.5 (4.2)	82.1 (6.7)	<0.001[a]
Gender – Females % (N)		38.8 (31)	50.0 (12)	33.3 (5)	34.1 (14)	0.400[b]
Ethnicity – % (N)	White British	93.8 (75)	95.8 (23)	100 (15)	90.2 (37)	0.727[b]
	White Irish	2.5 (2)	0 (0)	0 (0)	4.9 (2)	
	Indian	2.5 (2)	4.2 (1)	0 (0)	2.4 (1)	
	Arab	1.3 (1)	0 (0)	0 (0)	2.4 (1)	
Frailty index – mean (SD)		0.27 (0.11)	0.20 (0.09)	0.25 (0.13)	0.32 (0.09)	<0.001[a]
Clinical Frailty Scale – median (IQR)		4 (3 – 5)	3 (3 – 4)	3 (3 – 4)	5 (4 – 5)	<0.001[c]

[a]One-way ANOVA; [b]Chi-squared test; [c]Kruskal-Wallis test

4.2.4.1 Dynamic changes in frailty status

Frailty index did not change significantly across timepoints (Figure 4.2-1a; Table 8.4-4, Appendix 8.4). However, medical patients had higher FIs at baseline compared to patients within the surgical groups. There were significant differences in CFS across visits considering both changes in median values (Table 8.4-5, Appendix 8.4) and EMMs (Figure 4.2-1b; Table 8.4-6, Appendix 8.4). Similar to FI, CFS was higher for medical patients at baseline. The prevalence of Fried frailty significantly increased at 7 days post-operatively in the elective and emergency surgery groups (Figure 4.2-1c; Table 4.2-2). The prevalence of Fried frailty did not differ significantly across visits in the medical group, although the prevalence of Fried frailty was high at baseline in this group.

Table 4.2-2 – Frailty and sarcopenia prevalence separated by group and timepoint.
p values were derived from chi-squared tests; p<0.05 are denoted with *

	Baseline – % (N)	7 days – % (N)	13 weeks – % (N)	p value
Frailty Index				
Overall	61.3 (49/80)	75.0 (51/68)	67.9 (36/53)	0.204
Elective	33.3 (8/24)	54.5 (12/22)	52.4 (11/21)	0.281
Emergency	53.3 (8/15)	69.2 (9/13)	50.0 (5/10)	0.586
Medical	80.5 (33/41)	90.9 (30/33)	90.9 (20/22)	0.336
p value	0.001*	0.008*	0.010*	
Clinical Frailty Scale				
Overall	31.3 (25/80)	60.9 (42/69)	36.2 (21/58)	0.001*
Elective	4.2 (1/24)	50.0 (11/22)	14.3 (3/21)	0.001*
Emergency	13.3 (2/15)	53.8 (7/13)	40.0 (4/10)	0.071

Medical	53.7 (22/41)	70.6 (24/34)	51.9 (14/27)	0.230
p value	<0.001*	0.258	0.026*	
Fried Frailty				
Overall	60.0 (48/80)	84.8 (56/66)	57.5 (23/40)	0.001*
Elective	25.0 (6/24)	76.2 (16/21)	64.7 (11/17)	0.001*
Emergency	53.3 (8/15)	78.6 (11/14)	20.0 (2/10)	0.018
Medical	82.9 (34/41)	93.5 (29/31)	76.9 (10/13)	0.240
p value	<0.001*	0.176	0.017*	
Sarcopenia				
Overall	50.6 (40/79)	59.1 (39/66)	45.0 (18/40)	0.339
Elective	33.3 (8/24)	57.1 (12/21)	35.3 (6/17)	0.220
Emergency	57.1 (8/14)	61.5 (8/13)	40.0 (4/10)	0.565
Medical	58.5 (24/41)	59.4 (19/32)	61.5 (8/13)	0.982
p value	0.126	0.967	0.335	

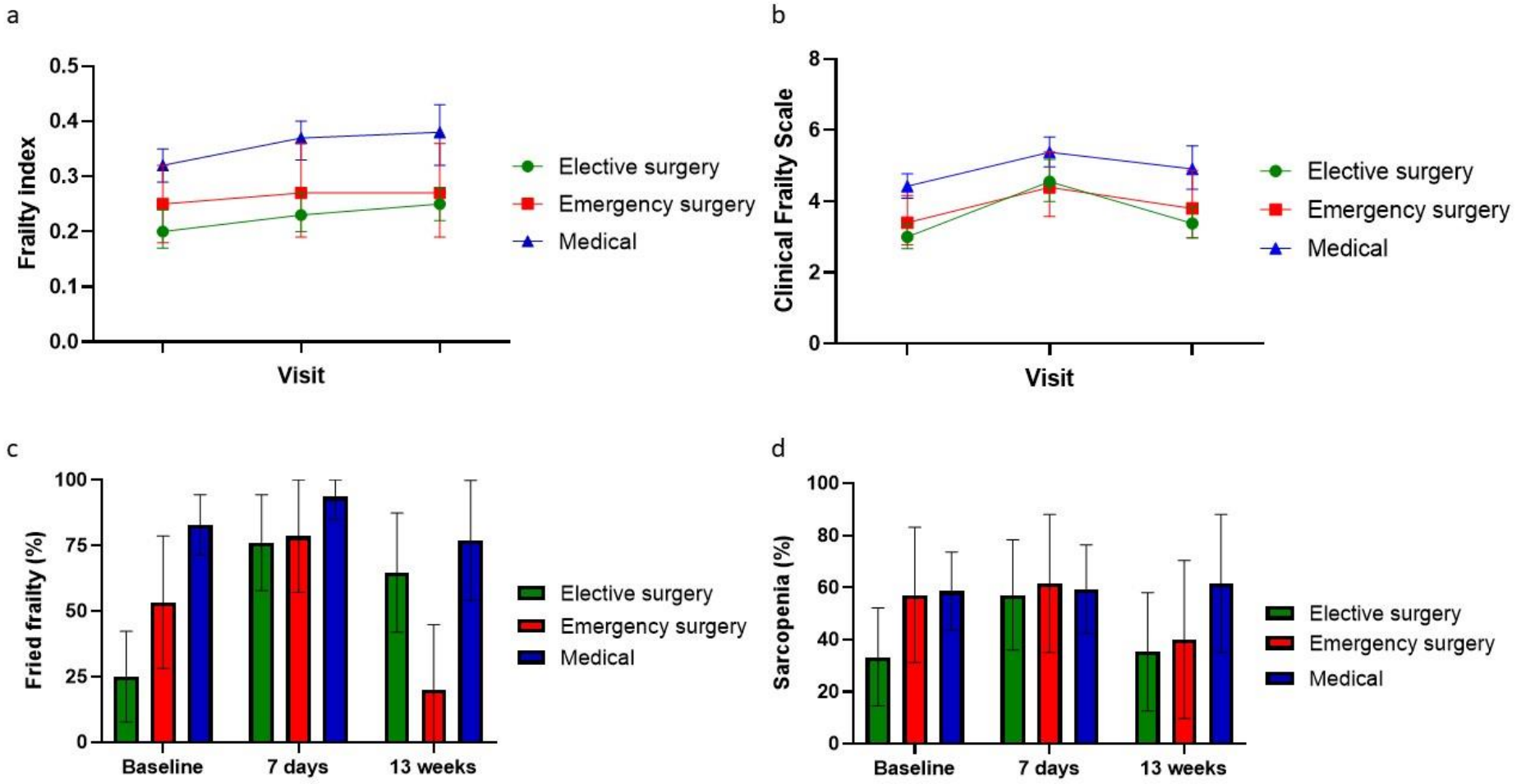

Figure 4.2-1 – Changes in frailty and sarcopenia status separated by cohort.

Clinical Frailty Scale increased from baseline at 7 days post-admission/post-operatively and returned towards baseline at 13 weeks for all groups. The prevalence of Fried frailty increased at 7 days in elective and emergency surgery patients. Frailty index and sarcopenia prevalence did not significantly differ across timepoints.

4.2.4.2 Dynamic changes in sarcopenia status

The prevalence of sarcopenia did not significantly differ across timepoints or between groups when considering sarcopenia as a binary construct (Figure 4.2-1d; Table 4.2-2). There were significant differences in ordinal categories of severity across timepoints for the study population overall; these differences were statistically significant when using a simulated Skillings-Mack model to account for ties (Table 8.4-7, Appendix 8.4). However, these differences were accounted for by participants meeting criteria for severe criteria at 7 days, where this had been unclear at baseline. Figure 4.2-2 demonstrates changes in sarcopenia status across timepoints. Of those who did not meet criteria for sarcopenia at baseline, 20.0% (5/25) (excluding drop-outs) met criteria for sarcopenia at 7 days, and a further 8.0% (2/25) had probable sarcopenia. Whilst some participants moved from lower sarcopenia status/severity to higher severity, others showed improvements in status to lesser severity. Four participants changed from severe sarcopenia to no sarcopenia at 7 days; two of these experienced a 1kg increase in handgrip strength, whereas the other two experienced 6kg and 10kg increases respectively. Individual change scores in components included within sarcopenia criteria from baseline to 7 days, and 7 days to 13 weeks are shown in Figure 8.4-3. Mean BATT and gait speed declined from baseline to 7 days, with a mean improvement/recovery from 7 days to 13 weeks. However, with all variables, there was considerable variation, with some participants experiencing declines in measurements between timepoints, and others experiencing improvements.

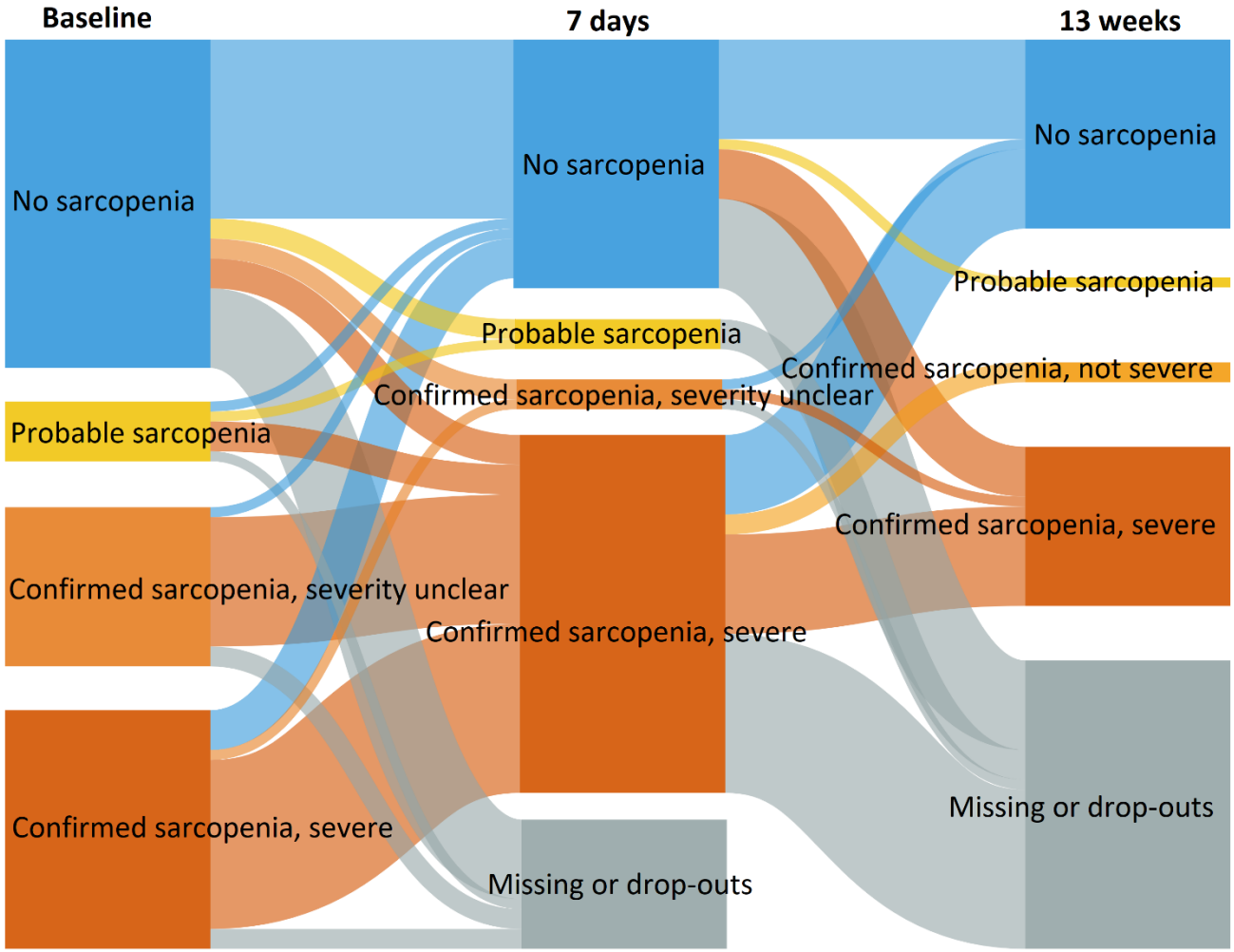

Figure 4.2-2 – Prevalence of sarcopenia status across timepoints.

The individual sections shown are proportional to the number of participants at each stage. Some participants experienced improvements in sarcopenia status, whereas others experienced worsening.

4.2.4.3 Changes in overlapping frailty and sarcopenia prevalence across timepoints

Figure 4.2-3 shows changes in frailty and sarcopenia prevalence across timepoints. Of all diagnostic criteria, CFS appeared the most discriminatory, with few participants meeting criteria for CFS alone at any timepoint. The least discriminatory was FI frailty. The proportion of participants meeting criteria for all frailty diagnoses and sarcopenia was greatest at the 7 day timepoint. Change in FI moderately correlated with change in CFS between baseline and 7 days (r_s=0.43; p<0.001), and 7 days and 13 weeks (r_s=0.37; p=0.018). Change in sarcopenia status did not correlate with change in FI or CFS between timepoints (Table 8.4-8 and Table 8.4-9, Appendix 8.4).

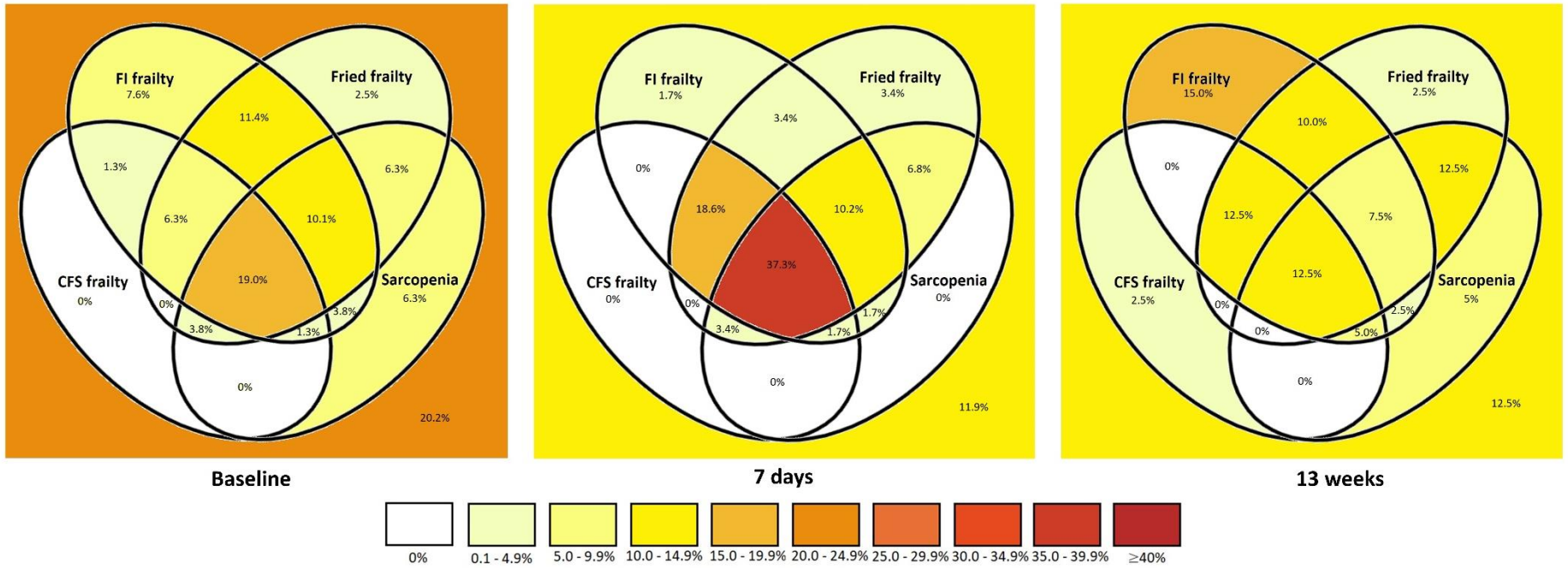

Figure 4.2-3 – Overlapping frailty and sarcopenia prevalence at each timepoint.

Areas of overlap with higher prevalence are colour-coded more red, with lower prevalent areas appearing yellow or lighter. Participants were included if all four criteria were available at the particular timepoint.

4.2.5 Discussion

Our results demonstrate that hospitalisation is associated with induced frailty in medical and surgical patients, where frailty is defined by Fried criteria or CFS. This effect was more marked in surgical patients, as medical patients already had higher rates of frailty at baseline. Importantly, this effect appears to be potentially reversible, with rates and severity of frailty returning towards baseline after 13 weeks. Dynamic changes in FI were less significant. This is to some extent unsurprising when considering the deficits that were included within the FI. The deficits that were included were validated from large community populations, and predominantly represent deficits that are chronic and acquired over time (Clegg et al., 2016). Dynamic changes may be more marked if deficits are modelled from a secondary care population, where risk is more likely to be affected by acutely evolving factors. Induced frailty is considered different to age-related frailty that progresses over time. Specifically, it occurs secondary to an insult, and our results are promising in demonstrating that this state is more likely to be temporary and reversible. However, even accepting this reversibility, it is potentially associated with equivalent individual risk in the short-term (Hawkins et al., 2018). Frailty has been shown to be associated with impairments in the immune system (Wilson et al., 2020). This will lead to a state of increased vulnerability, and increased likelihood of further deterioration in the event of secondary insults, potentially leading to a vicious cycle of heightened risk. This may be associated with increased risk of organ insufficiency, such as the risk of acute sarcopenia and muscle dysfunction through ineffective repair mechanisms (Welch et al., 2018).

Conversely, the prevalence of sarcopenia did not clearly change over time. However, examining individual trajectories demonstrated that some participants experienced improvements in their sarcopenia status, whereas others experienced declines. The significant overlap between sarcopenia and frailty at 7 days suggests that most participants who experienced acute sarcopenia also met criteria for frailty at this timepoint. Overall, significant overlap between diagnoses was demonstrated. Few people met criteria for just one frailty or sarcopenia diagnosis at any one time. The greatest overlap was observed at 7 days, with over a third of participants meeting criteria for all diagnoses. Considering the individual diagnoses, CFS was shown to be the most discriminatory, with few or no participants meeting criteria for frailty based on CFS alone at this timepoint. This suggests that if only one tool is to be used to assess vulnerability in clinical practice, then CFS may be the most pragmatic. The CFS is not specific to deficits in muscle or physical function, but similar to FI, encompasses a broader picture including cognition and other deficits (Rockwood and Mitnitski, 2007). The differences in overlap may also relate to the cut-offs that were used in defining frailty and sarcopenia. It is recognised that frailty forms a spectrum of increasing risk and vulnerability. Although a score of 5 or greater was selected, a CFS score of 4 is now considered to represent living with very mild frailty (Rockwood and Theou, 2020). Similarly, two participants changed from severe sarcopenia to no sarcopenia at 7 days, due to a 1kg improvement in handgrip strength, which is unlikely to represent clinically meaningful improvement.

4.2.5.1 Results in context of wider literature

Induced frailty is a relatively new concept. Previous studies have often evaluated frailty at a single timepoint, rather than as a dynamic construct. Where dynamic changes have been measured, this has normally been as part of a longitudinal study, rather than in the context of hospitalisation. However, longitudinal studies in community-dwelling older adults have shown that whilst some individuals will experience deteriorations in frailty status, some will not change, and others will experience improvement in frailty status (Li et al., 2021). A previous prospective study in Italy showed that of those without sarcopenia at admission, 14.7% of the sample developed sarcopenia during admission (Martone et al., 2017). These findings are not inconsistent with our study findings, as the incidence of sarcopenia at 7 days was 20.0% for those who did not have sarcopenia at baseline. However, we also showed that changes were bidirectional, to the extent that the overall prevalence did not change significantly between timepoints. Previous studies have demonstrated that there is overlap between Fried frailty and sarcopenia (Gingrich et al., 2019), and Fried frailty and FI, with differences in overlap dependent on cut-offs and definitions used (Li et al., 2015). The CFS is known to correlate with FIs, and was validated from the original study population (Rockwood and Mitnitski, 2007). The CFS is now the most common tool utilised in frailty assessment embedded into clinical practice. It has shown wide utility across a number of different clinical settings in predicting adverse outcomes and enabling holistic decision-making (Geriatric Medicine Research Collaborative and Covid Collaborative, 2021, Hewitt et al., 2019). At present, CFS is routinely measured for hospitalised older adults at the point of admission, based on function two weeks prior to this, but is not recorded dynamically during hospitalisation, or at the point of discharge. The Hierarchical Assessment of Balance and

Mobility is a tool that can be used to monitor progress and changes in in-bed mobility, transfers, and ambulation, in a similar manner to vital signs. Embedding of such tools into clinical practice could enable the identification of at risk individuals by monitoring trends over time (MacKnight and Rockwood, 1995).

4.2.5.2 Strengths and limitations

This study represents the first of its kind, prospectively characterising rates of frailty and sarcopenia across multiple timepoints in medical and surgical patients. We used recognised, validated diagnostic criteria in this process. Importantly, all recruitment and follow-up assessments were performed by clinicians with geriatric medicine expertise. However, there are a number of limitations that should be considered. Firstly, although the assessor did not refer back to assessments at earlier timepoints when performing frailty and sarcopenia assessments, they were not truly blinded to these readings. Secondly, considering sarcopenia diagnosis, we did not include measurements of muscle quality. Low muscle quality without low muscle quantity is now recognised as sufficient to meet criteria for sarcopenia. Echogenicity was recorded as part of this study, and we previously demonstrated that changes over 13 weeks correlated with change in handgrip strength and gait speed. However, at present there are no recognised cut-off values for sarcopenia that could be utilised. As echogenicity is known to vary between ultrasound devices, cut-offs would need to be validated from a reference population using the same device.

Thirdly, due to the COVID-19 pandemic, the sample size was smaller than that originally stated in the protocol. Our post-hoc power calculation demonstrates that the sample size achieved was sufficient to detect statistically significant differences in CFS frailty and Fried frailty prevalence at 7 days. However, we acknowledge that this sample size may have been insufficient to detect statistically significant differences in smaller changes in prevalence across timepoints. The rates of missing data were high, particularly at the 13 week timepoint, where it was not possible to perform in person assessments. It is unclear how this might have affected the results, but it will have reduced the overall power given the relatively small sample size. Lastly, importantly, this study is the first of its kind and provides proof of concept results that will need to be validated in a larger study across multiple settings. Frailty (measured by CFS (Church et al., 2020), FI (Tew et al., 2021), and Fried (Tew et al., 2021)), and sarcopenia (Bertschi et al., 2021, Martone et al., 2017) status have been measured with widespread use in hospitalised patients, although the FI variables used within this study were validated from a community population (Clegg et al., 2016). It is increasingly recognised that dynamic assessments are important, and studies have individually utilised these assessments both at admission to (Church et al., 2020, Bertschi et al., 2021, Martone et al., 2017), and discharge (Tew et al., 2021, Martone et al., 2017) from hospital.

4.2.5.3 Recommendations for future research and clinical practice

We consider that further research is warranted to determine what factors are predictive of changes in muscle quantity and function, and, importantly, the significance of such changes, before these assessments are in embedded into clinical practice. Research should focus on

determining what is different about those who experienced improvements in muscle quantity and function compared to those who experienced declines. Assessment of frailty status should occur at baseline for hospitalised patients; assessment of baseline CFS is normally recommended to consider function two weeks prior to admission to hospital to account for the effects of acute illness (Rockwood and Theou, 2020). However, frailty should be recognised and considered as a dynamic process. For instance, in patients with prolonged lengths of hospital stay, it may be appropriate to reassess frailty status, as it is likely that their vulnerability will have changed. This may have implications upon their overall management and goal-setting. Further research that aims to understand the effects of induced frailty upon immune dysregulation is strongly encouraged. The broad dynamic changes encountered in this study beyond changes in muscle and physical function alone have implications on how rehabilitation and interventional strategies are designed and implemented. Further research should address the benefits of multi-modal programmes e.g. targeting cognition as well as physical function.

4.2.6 Conclusion

Induced frailty and acute sarcopenia are overlapping conditions affecting older adults during hospitalisation. Induced frailty is likely to be reversible, but will be associated with increased vulnerability. Clinicians should be aware of the dynamic nature of frailty and sarcopenia, and should consider reassessing prior to discharge, and throughout admission in patients with prolonged lengths of stay. Further research should aim to stratify changes to enable targeted interventions.

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5 Mechanisms of acute sarcopenia

Chapter 5.1 – Associations of baseline nutritional status and in-hospital step count with muscle quantity, quality, and function: results of a prospective cohort study

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Submitted

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5.1 Associations of baseline nutritional status and in-hospital step count with muscle quantity, quality, and function: results of a prospective cohort study

5.1.1 Abstract

Seventy-nine participants aged ≥70 years (mean age 79.1 years, 44.3% female) were recruited from a UK university hospital. Elective colorectal surgery, emergency abdominal surgery, and general medical patients with infections were included. Baseline nutrition was assessed using the Mini-Nutritional Assessment. In-hospital step count was measured using Fitbit Inspire devices. Muscle quantity and quality (ultrasound quadriceps and bioelectrical impedance analysis), and physical function measurements were obtained at baseline, and 7(+/-2) days and 13(+/-1) weeks post-admission/post-operatively. Baseline nutritional status was significantly associated with rectus femoris ultrasound echogenicity (Normal: 58.5, At risk: 68.5, Malnourished: 81.2; p=0.025), Bilateral Anterior Thigh Thickness (Normal: 5.07cm, At risk: 4.03cm, Malnourished: 3.05cm; p=0.021), and Skeletal Muscle Mass (SMM) (Sergi equation) (Normal: 21.6kg, At risk: 18.2kg, Malnourished: 12.0kg; p=0.007). Step count was associated with baseline patient-reported physical function (<900 – 37.1, ≥900 44.5; p=0.010). There was a significant interaction between nutrition, step count, and time for SMM (Janssen equation) (p=0.022).

5.1.2 Background

Acute sarcopenia (acute muscle insufficiency) is defined by the development of incident sarcopenia (low muscle strength with low muscle quantity and/or quality) within six months, normally following a stressor event (Welch et al., 2018, Cruz-Jentoft et al., 2019). It is increasingly recognised as a target for therapeutic trials (Welch et al., 2020b). Sarcopenia is known to impact upon quality of life (Beaudart et al., 2015), and prevalence increases with age (Cruz-Jentoft et al., 2019, Dodds et al., 2014). Trials that demonstrate benefit, therefore, have potential to dramatically improve the lives of older adults following hospitalisation. Mean step count over up to three consecutive days during hospitalisation has previously been shown to be associated with functional decline from premorbid function (two weeks prior to hospitalisation) to discharge (Agmon et al., 2017). However, the benefits of physical activity upon skeletal muscle can be blunted when nutrition, particularly protein intake, is inadequate (Shad et al., 2016). The interactions of the effects of nutrition and step count with dynamic changes in skeletal muscle in hospitalised older adults have not previously been characterised. This study aimed to assess the associations between in-hospital step count and baseline nutritional status with muscle quantity, quality, and function measurements.

5.1.3 Methods

5.1.3.1 Study design and setting

The protocol for the main study has been published previously (Welch et al., 2020a) and the

study was prospectively registered (NCT03858192). Participants were recruited from a single centre, the Queen Elizabeth Hospital Birmingham. Three groups of participants aged 70 years and older were recruited: patients expected to undergo elective colorectal surgery, patients who had undergone emergency abdominal surgery, and general medical patients with acute infectious diseases. Participants were excluded if they were considered to be imminently approaching the end of life, or if they were unable to understand verbal or written English. Ethical approval was obtained from Wales Research Ethics Committee 4. Participants provided written informed consent or consultee declaration was obtained if they were deemed to lack capacity to consent for themselves. Baseline assessments were performed in preoperative assessment clinic in the elective cohort, within 48 hours of surgery in the emergency surgery cohort, and within 48 hours of admission in the medical cohort. Further assessments were performed at 7 (+/-2) days post-operatively/post-admission, and 13 (+/-1) weeks post-operatively/post-admission. Recruitment and assessments were performed by a clinician with training and expertise in geriatric medicine.

5.1.3.2 Muscle quantity and quality assessment

Muscle quantity and quality were assessed at each timepoint using quadriceps ultrasound and Bioelectrical Impedance Analysis (BIA) (Welch et al., 2020a). Bilateral Anterior Thigh Thickness (BATT) was calculated as the total thickness of the right and left rectus femoris and vastus intermedius muscles, measured at the midpoint between the greater trochanter and lateral joint line in the transverse plane using a linear probe (Venue 50, GE Healthcare) (Wilson et al., 2019). Rectus femoris echogenicity was measured using longitudinal images taken at

the same location by grey scale analysis using Image J software (Wilson et al., 2019). Skeletal Muscle Mass (SMM) was calculated from BIA measurements (Bodystat Quadscan 4000) using two previously validated equations: SMMJanssen (Janssen et al., 2000), and SMMSergi (Sergi et al., 2015) (Table 8.5-1, Appendix 8.5).

5.1.3.3 Physical function assessment

Handgrip strength was measured at each timepoint using a Jamar dynamometer. Participants were advised to squeeze as hard as they were able to, and the best result from two measures on each side was used in analysis (Roberts et al., 2011). Usual gait speed was measured at each timepoint across a four metre course, by advising participants to walk at a "comfortable pace" (Rydwik et al., 2012), except for emergency surgery patients at baseline assessment, where this was not possible. Patient-reported physical function was assessed using the Patient-Reported Outcomes Measurements Information System (PROMIS®) item bank V2.0 Physical Function Short Form 10b questionnaire at each timepoint (Tatsuoka et al., 2016). Raw scores were entered into the HealthMeasures Scoring Service, powered by Assessment Center℠ to derive T-scores.

5.1.3.4 Nutritional assessment

Baseline nutritional assessment was performed using the Mini Nutritional Assessment (Full Form) (MNA) (Vellas et al., 1999). Participants were categorised as normal (24 to 30 points), at risk of malnutrition (17 to 23.5 points), or malnourished (Less than 17 points).

5.1.3.5 Step count

Step count was measured during the inpatient stay from initial post-operative/post-admission assessment to 7 (+/-2) days post-operatively using Fitbit Inspire devices worn on the non-dominant wrist. This was included as an optional aspect of the study. Mean daily step count was calculated from days where whole day data was available (i.e. excluding the days that the device was applied or removed for data extraction). Participants were advised to wear the device all the time, but were able to remove the device when washing or sleeping for comfort. Participants were categorised as having <900 or ≥900 steps/day as per previously validated cut-offs for functional decline (Agmon et al., 2017).

5.1.3.6 Frailty assessment

Frailty was assessed at each timepoint using both a frailty index (Rockwood and Mitnitski, 2007) and Clinical Frailty Scale (CFS) (Pulok et al., 2020). The variables included within the frailty index were adapted from those previously validated within the electronic Frailty Index (eFI), which was developed from a UK community population (Clegg et al., 2016). The CFS was assessed by a clinician with expertise in geriatric medicine, based on function two weeks prior to admission. All information from a full comprehensive assessment including Katz (Katz et al., 1963) and Lawton (Lawton and Brody, 1969) Activities of Daily Living (ADLs) were used when assessing the CFS.

5.1.3.7 Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics Version 26 (IBM Corp). This study represents a substudy of the main study. The study was initially powered to enable detection of clinically significant differences in muscle and physical function measurements within groups (45 to follow-up in each group). In light of recruitment to the study being paused during the Coronavirus 2019 (COVID-19) pandemic, the recruitment target was revised to enable detection of differences across groups (45 to follow-up across groups). Descriptive statistics were summarised at baseline according to categories of nutrition and step count. Statistical significance of differences were analysed using Analysis of Covariance (ANOVA) (nutrition) or independent samples t-tests (step count) for parametric continuous variables, Kruskal-Wallis (nutrition) or Mann-Whitney U tests (step count) for non-parametric continuous or ordinal variables, and chi-squared tests for proportions. Statistical significance of differences in muscle quantity, quality, and physical function measurements between nutrition and step count groups, and between timepoints were analysed using Linear Mixed Models (parametric variables) or Generalized Linear Mixed Models (non-parametric variables). Interaction terms for nutrition*timepoint, step count*timepoint, and nutrition*step count*timepoint were included within the models. Estimated marginal means across timepoints and groups were calculated from these models. Statistical significance was set at $p<0.050$.

5.1.4 Results

Eighty-one participants were recruited to this study. Two participants were excluded from this analysis (one elective admission in the emergency surgery cohort, one emergency surgery participant did not undergo surgery). Recruitment and drop-out rates are shown in Figure 8.5-1 (Appendix 8.5). Full feasibility analysis, screening and recruitment rates have been published previously (Welch et al., 2021a). Baseline characteristics are shown in Table 5.1-1. The mean age of participants was 79.1 (6.6), and 44.3% were female. There were no significant differences between nutrition or step count groups in terms of age, sex, or ethnicity of participants. However, a greater proportion of at risk or malnourished participants were medical patients and had greater frailty indices and CFS compared to those with normal nutrition. Participants with lower step counts also had higher CFS at baseline.

5.1.4.1 Muscle quantity and quality

Table 5.1-2 demonstrates results of mixed models with estimated marginal means derived from interaction terms with timepoint. These results are shown graphically for muscle quantity and quality in Figure 5.1-1. Participants with greater risk of malnutrition had significantly lower BATT, SMMSergi, and SMMJanssen, and higher echogenicity across timepoints. Differences were statistically significant for SMMJanssen with a model including interaction terms for nutrition, step count, and timepoint together. These results are shown graphically in Figure 5.1-2. Visually analysing trends showed that the most severely malnourished participants continued to experience declines in BATT at 13 weeks across

timepoints, whereas those with normal or at risk nutrition recovered declines in BATT demonstrated at 7 days, although these changes were not statistically significant. Non-statistically significant lower BATT, SMMSergi, and SMMJanssen, and higher echogenicity were demonstrated for participants with lower step counts. Visually analysing trends, participants with lower step counts experienced more rapid declines in BATT at 7 days, allowing for baseline differences.

5.1.4.2 Muscle and physical function

No associations were demonstrated between nutrition and PROMIS T-score or gait speed. Handgrip strength was lower in participants with greater risk of malnutrition (Figure 5.1-3), although these differences were not statistically significant. Participants with lower step count had significantly lower PROMIS T-scores across all timepoints, but step count was not predictive of differences across timepoints. Lower handgrip strength and slower gait speeds were demonstrated in participants with lower step counts, but these differences were not statistically significant.

Table 5.1-1 – Baseline demographics of participants separated by nutritional status and step count

		Overall (N=79)	Nutrition				Step count		
			Normal (N=33)	At risk (N=40)	Malnourished (N=6)	p value	<900/ day (N=20)	≥900/ day (N=15)	p value
Age (years) – mean (SD)		79.1 (6.6)	77.6 (6.4)	80.7 (7.0)	77.7 (2.7)	0.114	79.7 (7.8)	75.9 (4.2)	0.098
Sex – %females (N)		44.3% (35)	42.4% (14)	42.5% (17)	66.7% (4)	0.518	50.0% (10)	40.0% (6)	0.557
Ethnicity – % (N)	White British	93.7% (74)	97.0% (32)	92.5% (37)	83.3% (5)	0.336	90.0% (18)	93.3% (14)	0.416
	White Irish	2.5% (2)	0% (0)	2.5% (1)	16.7% (1)		0% (0)	6.7% (1)	
	Indian	2.5% (2)	3.0% (1)	2.5% (1)	0% (0)		5% (1)	0% (0)	
	Arab	1.3% (1)	0% (0)	2.5% (1)	0% (0)		5% (1)	0% (0)	
Group – % (N)	Elective surgery	30.4% (24)	54.5% (18)	15.0% (5)	0% (0)	0.001*	30.0% (6)	40.0% (6)	0.826
	Emergency surgery	17.7% (14)	15.2% (5)	22.5% (9)	0% (0)		15.0% (3)	13.3% (2)	
	Medical	51.9% (41)	30.3% (10)	62.5% (25)	100%		55.0% (11)	46.7% (7)	
Body Mass Index (kg/m^2) – mean (SD)		26.5 (6.5)	28.4 (5.2)	25.8 (7.2)	20.4 (2.7)	0.010*	26.0 (7.8)	26.9 (5.2)	0.715
Baseline nutrition – % (N)	Normal	37.1% (13)	100% (33)	0% (0)	0% (0)	NA	30.0% (6)	46.7% (7)	0.135
	At risk	51.4% (18)	0% (0)	100% (40)	0% (0)		65.0% (13)	33.3% (5)	
	Malnourished	11.4% (4)	0% (0)	0% (0)	100% (6)		5.0% (1)	20.0% (3)	
Step count	Median (IQR)	708 (250 – 1690)	1028 (386 – 2366)	491 (180 – 951)	2023 (735 – 4454)	0.072	338 (95 – 642)	1812 (1232 – 2792)	<0.001
	<900 – % (N)	57.1% (20)	46.2% (6)	72.2% (13)	25.0% (1)	0.135	100% (0)	0% (0)	NA
	≥900 – % (N)	42.9% (15)	53.8% (7)	27.8% (5)	75.0% (3)		0% (0)	100% (0)	
Baseline frailty index – mean (SD)		0.27 (0.11)	0.22 (0.10)	0.31 (0.11)	0.33 (0.05)	0.001*	0.29 (0.10)	0.26 (0.11)	0.491
Baseline Clinical Frailty Scale – median (IQR)		4 (3 – 5)	3 (2.5 – 3)	4.5 (4 – 5)	4.5 (3.75 – 5.25)	<0.001*	5 (3 – 5)	3 (3 – 4)	0.006*

Table 5.1-2 – Estimated marginal means with 95% confidence intervals derived from mixed models for nutritional status and step count across timepoints.

		Nutrition			*p value*		Step count		*p value*		
		Normal	At risk	Malnourished	*Nutrition*	*Nutrition* Timepoint*	<900/ day	≥900/ day	*Steps*	*Steps* Timepoint*	*Steps* Nutrition* Timepoint*
BATT (cm)	Baseline	5.07 (4.33 – 5.82)	4.03 (3.33 – 4.73)	3.05 (1.51 – 4.60)	*0.021**	*0.741*	3.86 (2.87 – 4.86)	4.24 (3.51 – 4.97)	*0.315*	*0.543*	*0.909*
	7 days	4.66 (3.95 – 5.37)	3.82 (3.15 – 4.49)	2.71 (1.24 – 4.18)			3.29 (2.34 – 4.24)	4.17 (3.47 – 4.87)			
	13 weeks	5.05 (4.17 – 5.93)	4.10 (3.26 – 4.94)	2.39 (0.53 – 4.25)			3.59 (2.43 – 4.74)	4.11 (3.17 – 5.04)			
Echogenicity	Baseline	58.5 (51.0 – 65.9)	68.5 (61.3 – 75.7)	81.2 (65.7 – 96.8)	*0.025**	*0.461*	71.4 (61.4 – 81.4)	67.4 (60.0 – 74.8)	*0.466*	*0.989*	*0.065*
	7 days	58.2 (51.6 – 64.8)	65.0 (58.1 – 72.0)	75.2 (61.4 – 89.0)			67.6 (58.7 – 76.5)	64.6 (57.9 – 71.3)			
	13 weeks	62.7 (54.6 – 70.8)	71.8 (64.1 – 79.6)	73.2 (56.7 – 89.8)			71.1 (61.0 – 81.1)	67.5 (58.7 – 76.3)			
SMMSergi (kg)	Baseline	21.6 (18.9 – 24.3)	18.2 (15.6 – 20.7)	12.0 (6.6 – 17.5)	*0.007**	*0.363*	16.2 (12.6 – 19.7)	18.3 (15.7 – 20.9)	*0.302*	*0.472*	*0.265*
	7 days	22.0 (19.0 – 25.0)	18.0 (15.3 – 20.8)	11.8 (5.8 – 17.7)			16.4 (12.6 – 20.3)	18.1 (15.3 – 21.0)			
	13 weeks	22.3 (19.5 – 25.1)	17.3 (14.5 – 20.0)	13.1 (7.4 – 18.8)			16.0 (12.4 – 19.7)	19.0 (16.2 – 21.9)			
SMMJansen (kg)	Baseline	25.0 (21.2 – 29.5)	20.6 (17.6 – 24.0)	13.4 (9.6 – 18.7)	*0.004**	*0.209*	18.1 (14.6 – 22.5)	20.0 (17.0 – 23.4)	*0.369*	*0.164*	*0.022*
	7 days	25.8 (21.3 – 31.4)	20.0 (16.7 – 23.9)	12.3 (8.4 – 18.1)			17.8 (13.8 – 22.8)	19.3 (16.0 – 23.3)			

	13 weeks	24.6 (21.1 – 28.7)	19.2 (16.6 – 22.2)	13.9 (10.2 – 19.1)			17.1 (14.0 – 20.9)	20.5 (17.6 – 23.9)			
PROMIS	Baseline	45.9 (41.5 – 50.3)	38.9 (34.7 – 43.0)	37.7 (28.6 – 46.8)	*0.145*	*0.081*	37.1 (13.2 – 43.0)	44.5 (40.2 – 48.8)	*0.010**	*0.988*	*0.848*
	7 days	38.0 (33.6 – 42.4)	35.7 (31.4 – 40.1)	38.3 (29.1 – 47.5)			33.6 (27.6 – 39.5)	41.1 (36.7 – 45.6)			
	13 weeks	45.30 (39.9 – 50.7)	38.7 (33.9 – 43.6)	51.1 (39.2 – 63.0)			38.0 (33.2 – 42.7)	47.7 (42.4 – 53.1)			
Handgrip strength (kg)	Baseline	20.8 (15.3 – 26.2)	17.7 (12.6 – 22.9)	13.7 (2.4 – 25.0)	*0.285*	*0.598*	15.8 (8.6 – 23.1)	19.0 (13.6 – 24.3)	*0.357*	*0.845*	*0.977*
	7 days	21.5 (16.3 – 26.7)	16.1 (11.2 – 21.0)	13.5 (2.8 – 24.3)			15.1 (8.2 – 22.0)	19.0 (13.8 – 24.1)			
	13 weeks	24.9 (19.1 – 30.7)	19.9 (14.3 – 25.5)	17.3 (4.9 – 29.7)			18.4 (10.8 – 25.9)	23.1 (16.7 – 29.4)			
Gait speed (m/s)	Baseline	0.71 (0.58 – 0.87)	0.70 (0.55 – 0.89)	0.47 (0.31 – 0.69)	*0.498*	*0.202*	0.58 (0.45 – 0.76)	0.64 (0.53 – 0.79)	*0.262*	*0.734*	*0.485*
	7 days	0.52 (0.39 – 0.69)	0.47 (0.31 – 0.69)	0.62 (0.35 – 1.08)			0.47 (0.32 – 0.68)	0.60 (0.46 – 0.80)			
	13 weeks	0.72 (0.54 – 0.97)	0.53 (0.40 – 0.71)	0.52 (0.28 – 0.95)			0.53 (0.36 – 0.77)	0.65 (0.47 – 0.89)			

BATT=Bilateral Anterior Thigh Thickness; SMMSergi=Skeletal Muscle Mass (Sergi equation); PROMIS=Patient-Reported Outcomes Measurements Information System, T-Score for physical function

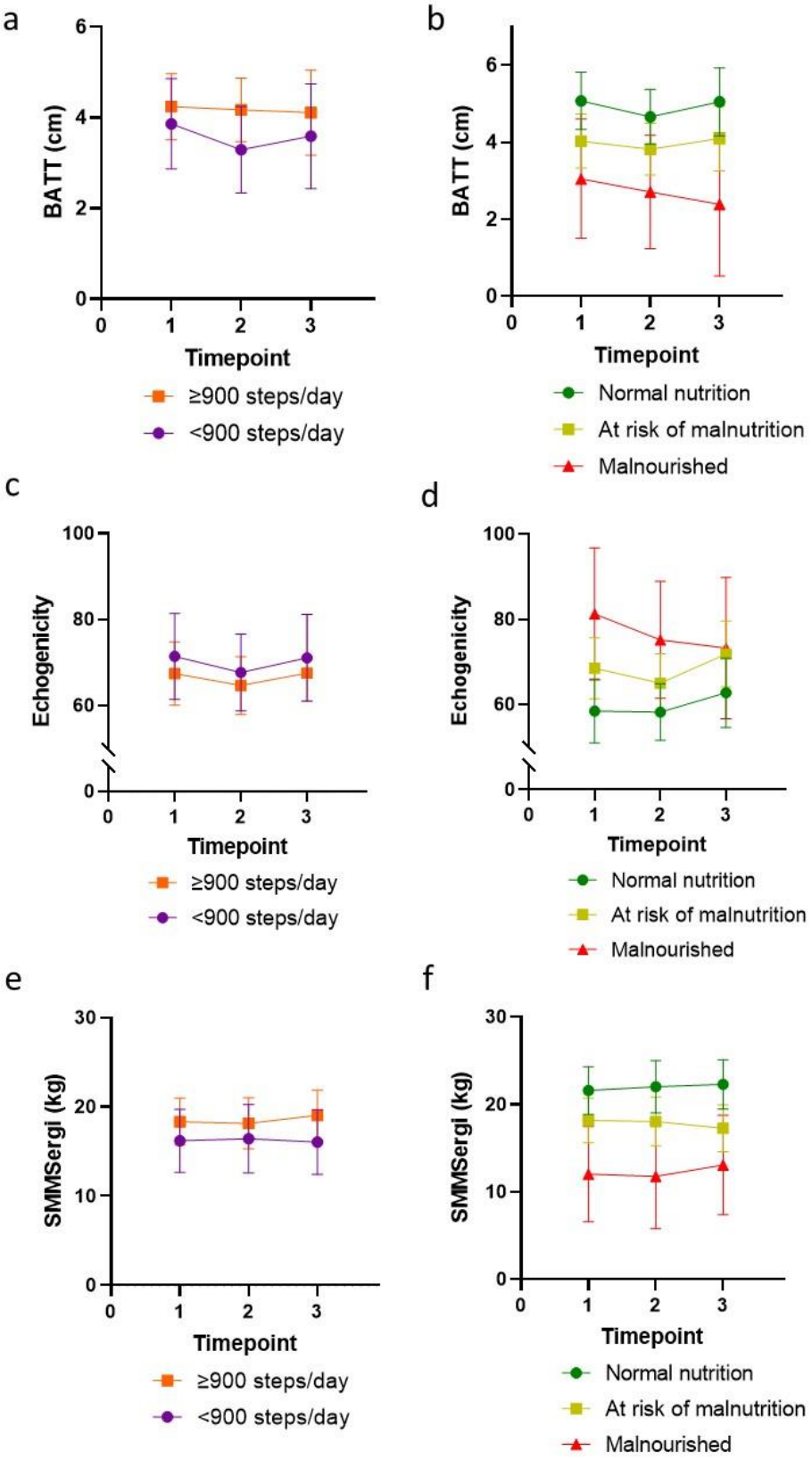

Figure 5.1-1– Estimated marginal means of muscle quantity and quality measurements according to nutritional status and step count.

BATT=Bilateral Anterior Thigh Thickness, SMMSergi=Skeletal Muscle Mass (Sergi equation)

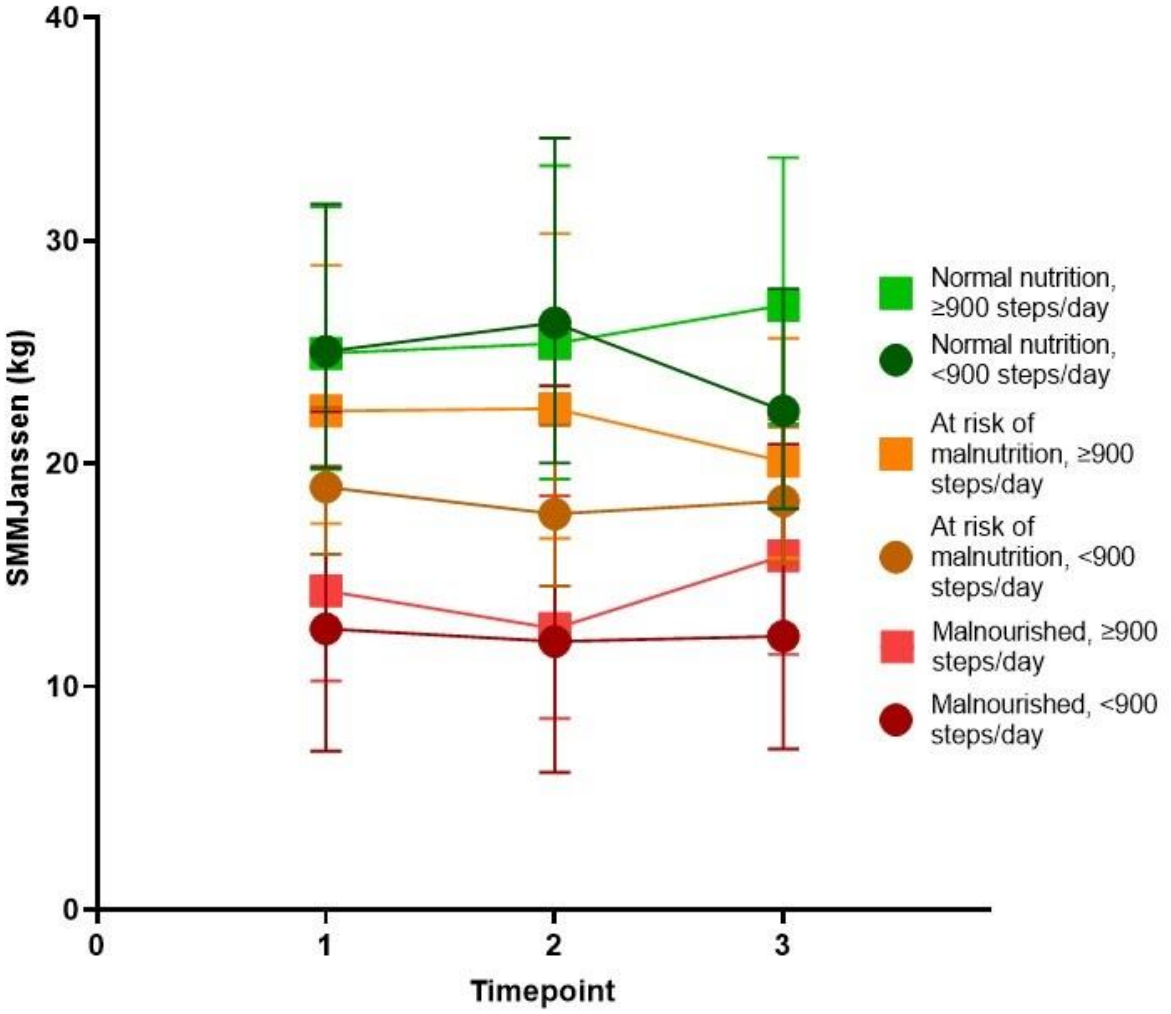

Figure 5.1-2 – Estimated marginal means of SMMJanssen separated by nutritional status and step count.

SMMJanssen=Skeletal Muscle Mass (Janssen equation)

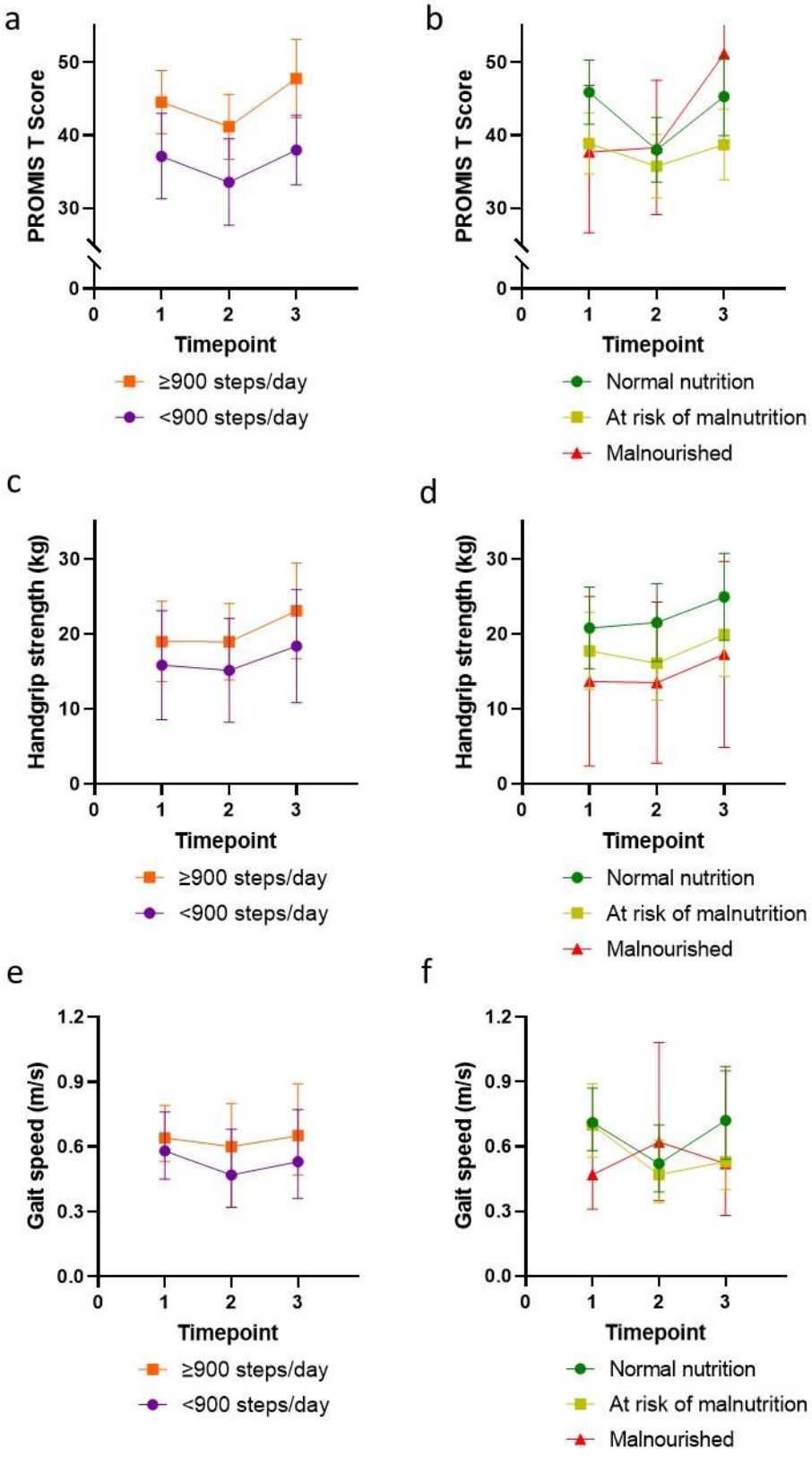

Figure 5.1-3 – Estimated marginal means of physical function measurements according to nutritional status and step count.

PROMIS=Patient-Reported Outcomes Measurements Information System, T-Score for physical function

5.1.5 Discussion

In this study, baseline nutritional status was associated with baseline muscle quantity (BATT, SMMSergi, SMMJanssen) and quality (low rectus femoris echogenicity), but was not conclusively predictive of change. This suggests that malnutrition is associated with the development of chronic sarcopenia, but the relationship with acute sarcopenia is unclear. There was some suggestion that the most malnourished participants were the least likely to recover acute losses in muscle quantity. However, nutritional status was not associated with physical function or performance, indicating that additional factors are involved in these pathways.

Conversely, step count was significantly associated with patient reported physical function, although this does not necessarily indicate causation. Step count itself could be considered a marker of physical function, rather than low step count being causative of declines in physical function. Although not statistically significant, muscle quantity, quality, and strength were consistently reduced with reduced step count, and there was suggestion of greater acute decline in BATT. This implies that a low step count in-hospital may be acutely detrimental regardless of baseline muscle size, and that step count may not simply be a marker of baseline function. There was a statistically significant interaction term between nutrition, step count, and timepoint for SMMJanssen, although there were no statistically significant differences for interaction terms with timepoint and either nutrition or step count alone. Low step count was associated with declines in muscle quantity with normal nutrition, whereas high step count was associated with recovery of muscle quantity with malnutrition.

5.1.5.1 Results in the context of other literature

These results are consistent with previous studies, which have demonstrated low fat-free mass measured by BIA in malnourished (defined by Subjective Global Assessment) hospitalised patients admitted with exacerbations of Chronic Obstructive Pulmonary Disease (Teixeira et al., 2020), and older hospitalised patients at high risk of malnutrition (defined by the Short Nutritional Assessment Questionnaire) (Pierik et al., 2017). The latter study included serial measurements and no change in muscle mass during hospitalisation was demonstrated regardless of nutritional status (Pierik et al., 2017).

The cut-off of 900 steps/day was previously demonstrated to predict decline in functional independence, as measured by the Barthel index and instrumental ADLs, during hospitalisation (Agmon et al., 2017). However, a subsequent study suggested that this cut-off may have high specificity but low sensitivity for hospital associated disability; lower step count was demonstrated in patients experiencing declines in ADLs (Pavon et al., 2020). A unit-tailored mobility programme with a specific goal of at least 900 steps/day was shown to be associated with a lesser decline in ADLs in a quasi-experimental study (Cohen et al., 2019).

Fourteen days of reduced steps in healthy older adults has been shown to be associated with declines in leg fat-free mass measured using Dual energy X-ray Absorptiometry (DXA). These declines were associated with reduced postprandial rates of muscle protein synthesis (Breen et al., 2013). This suggests that reduced step count is associated with anabolic resistance, thus, negative effects may be exacerbated if protein intake is especially low. This is consistent

with the results of our study, demonstrating an interaction between nutrition and step count in terms of change in SMMJanssen over time.

5.1.5.2 Limitations

We acknowledge that there are a number of important limitations related to this study. Firstly, the MNA provides an assessment of nutrition over the preceding three months (Vellas et al., 1999), and is less sensitive to acute illness related effects. We did not record nutritional intake during hospitalisation in this study, and it is unclear how this may have impacted upon dynamic changes. Secondly, we recognise that there are limitations in the use of Fitbit devices as opposed to raw accelerometery data. In older adults with very low step count, this may have led to a floor effect, and it was not possible to assess for the effects of differences in position (Heesch et al., 2018). Additionally, although step count provides a quantitative estimate of physical activity, it does not differentiate between resistance exercise, aerobic exercise, and simple mobilisation. Thirdly, no gold standard measure of muscle quantity (DXA, Magnetic Resonance Imaging, or Computer Tomography (Cruz-Jentoft et al., 2019)) was used in this study. Fluid balance affects estimation of skeletal muscle mass with BIA (Nakanishi et al., 2019) and may affect ultrasound measurements in the presence of significant oedema (Fischer et al., 2016). Ultrasound in particular can be affected by changes in position (Welch et al., 2021b). Fourth, due to recruitment challenges during the COVID-19 pandemic, it was not possible to recruit sufficient numbers of participants to meet the original sample size target. The number of participants who met criteria for being malnourished at baseline was particularly small, and confidence intervals were wide. This may explain why nutritional status was not associated with physical function/performance, as similar trends to other measures

were demonstrated in those with normal and at risk nutrition. Finally, there were higher rates of frailty and medical patients amongst participants who met criteria for malnutrition, and higher rates of frailty at baseline in participants with low step counts. The direction of causality is unclear for these associations.

5.1.5.3 Recommendations for clinical practice and future research

The identification of patients who are malnourished or at risk of malnutrition in hospital is important, and nutritional assessment should be incorporated into any Comprehensive Geriatric Assessment that includes assessment of frailty and sarcopenia status. Step count is not currently measured within routine clinical practice, but our study is confirmatory of previous studies, which have demonstrated that assessment of step count within a clinical environment is feasible. Routine use of physical activity monitors in hospital could allow real-time monitoring with trends over time, which could be viewed remotely in a similar manner to vital signs. Large community studies are currently underway to establish the effectiveness of protein supplementation and resistance exercise to prevent and treat chronic sarcopenia (Landi et al., 2017). Further research is warranted to establish the effectiveness of combined nutritional and physical activity interventions in hospitalised older adults.

5.1.6 Conclusion

Baseline nutritional status is associated with baseline muscle quantity and quality in hospitalised older adults. Baseline physical function is associated with reduced step count during hospitalisation. There is some suggestion that there may be interactions between the effects of nutritional status and physical activity in predicting dynamic changes in muscle quantity. Further research should aim to stratify and examine underlying mechanisms, to enable the development of targeted combined nutritional and physical activity interventions.

5.1.7 References

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Chapter 5.2 – Establishing biomarkers of acute sarcopenia: a proof-of-concept study utilising network analysis

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5.2 Establishing biomarkers of acute sarcopenia: a proof-of-concept study utilising network analysis

5.2.1 Abstract

Background: Acute sarcopenia is defined by the development of incident sarcopenia (i.e. low muscle strength with low muscle quantity or quality) within six months, normally following a stressor event. However, few studies have evaluated the relationship of systemic biomarkers with sarcopenia during hospitalisation.

Methods: Prospective observational study at a single university hospital in the UK, involving elective colorectal surgery, emergency abdominal surgery, and general medical patients with infections aged 70 years and older. Serial measurements were performed preoperatively within the elective group, and within 48 hours, 7 days after, and 13 weeks after admission or surgery. Muscle strength was defined by handgrip strength, and muscle quantity was defined by Bilateral Anterior Thigh Thickness (BATT) measured through ultrasound and/or Skeletal Muscle Mass (Sergi equation) (SMMSergi) derived from Bioelectrical Impedance Analysis. Rectus femoris echogenicity was also recorded as a marker of muscle quality. Serum and plasma samples were collected preoperatively in the elective group and within 48 hours of admission/surgery in all groups. Least Absolute Shrinkage and Selection Operator (LASSO) models were used to identify clinical features and systemic biomarkers associated with sarcopenia at 7 days, adjusting for baseline sarcopenia status, as well as change in BATT, SMMSergi, and echogenicity at 7 days. Each model was fitted and reported with variables available at each timepoint. Coefficients from models were used to generate networks.

Results: Seventy-nine participants were recruited to the study and included in this analysis (mean age 79.1, 39.2% female). Chronic Obstructive Pulmonary Disease (COPD) (48 hours β 0.67, CI 0.59 – 0.75), and prescription of steroids during admission (48 hours β 1.11, CI 0.98 – 1.24) were positively associated with sarcopenia at 7 days. The presence of delirium was negatively associated with change in BATT to 7 days (7 days β -0.47, CI -0.5 – -0.44). COPD (Preoperative β 0.35, CI 0.12 – 0.58) and delirium (48 hours β 0.13, CI 0.06 – 0.2) were positively associated with change in echogenicity to 7 days in analysis including systemic biomarkers. Participants who met criteria for sarcopenia at baseline had significantly higher IL-7 concentrations measured during the acute phase of illness (median 8.78pg/mL vs 6.52pg/mL; p=0.014). IL-1b measured within 48 hours of admission/surgery was positively associated with sarcopenia status at 7 days (β 0.24, CI 0.06 – 0.42).

Conclusions: Patients most at risk of acute sarcopenia or reductions in muscle quantity and quality included those prescribed steroids, with COPD or delirium, or with heightened systemic inflammation.

5.2.2 Introduction

Acute sarcopenia (acute muscle insufficiency (Cruz-Jentoft, 2016)), is recognised as an important emergent diagnosis, particularly affecting hospitalised older adults (Welch et al., 2018, Welch, 2021). It is defined by the development of incident sarcopenia (i.e. low muscle strength with low muscle quantity or quality) within six months, normally following a stressor event (Cruz-Jentoft et al., 2019). Recent studies have characterised changes in muscle quantity, quality and function in hospitalised populations (Hartley et al., 2020, Van Ancum et

al., 2017a, Van Ancum et al., 2017b), with further studies ongoing or proposed. However, few studies have evaluated the relationship of systemic biomarkers with the development of sarcopenia, or assessed how predisposing or precipitating factors may cluster to increase risk and enable treatment stratification. In chronic sarcopenia, biomarkers associated with sarcopenia prevalence include myostatin (Patel et al., 2014), inflammatory cytokines (Patel et al., 2014), and Growth Hormone (GH)/Insulin-like Growth Factor 1 (IGF-1). Delirium is an acute neuropsychiatric disorder that occurs commonly secondary to acute illness in older adults, and which is associated with systemic inflammation (Kealy et al., 2020). However, patients with delirium have been frequently excluded from trials of interventions to combat negative changes in muscle quantity and physical function in hospitalised older people (Welch et al., 2020b). This study aimed to enhance understanding of how time-dependent biomarkers and patient-related factors may relate to acute sarcopenia risk.

5.2.3 Methods

5.2.3.1 Study setting and design

This was a single centre study conducted at the Queen Elizabeth Hospital Birmingham, UK. The original protocol for the study was published previously (Welch et al., 2020) and the study was prospectively registered (NCT03858192). Participants aged 70 years and older were recruited to three groups: elective surgery (participants planned to undergo a major colorectal surgery procedure), emergency surgery (participants who had undergone an emergency abdominal procedure), and medical (admitted with acute bacterial infections or

Coronavirus 2019, COVID-19). Participants either provided informed consent or consultee declaration was obtained if they were considered to lack capacity to consent for themselves. Elective surgery participants were recruited from preoperative assessment clinic, and emergency surgery and medical participants were recruited from surgical and medical wards respectively. Exclusion criteria included inability to understand written or verbal English, and life expectancy less than 30 days.

5.2.3.2 Research procedures

Baseline assessments were performed in preoperative assessment clinic in elective surgery participants, within 48 hours of surgery in emergency surgery participants, and within 48 hours of admission in medical participants. All assessments were performed by a clinician with training and expertise in geriatric medicine. Further assessments were performed at 48 hours post surgery in the elective group, 7 (+/-2) days post-admission/ post-operatively, and 13 (+/-1) weeks post-admission/ post-operatively. Assessments performed at each timepoint included handgrip strength, ultrasound quadriceps, and Bioelectrical Impedance Analysis (BIA). Ultrasound (Venue 50, GE Healthcare) quadriceps was performed on both sides at the midpoint between the greater trochanter at the hip and the lateral joint line of the knee. The thickness of the rectus femoris (RF) and vastus intermedius (VI) muscles was measured on serial images not including the fascia in the transverse plane. The average of each thickness measurement was used for analysis. Bilateral Anterior Thigh Thickness (BATT) was calculated as the total thickness of the right RF + right VI + left RF + left VI (Welch et al., 2020, Wilson et al., 2019). A single image was taken on each side in the longitudinal plane and RF echogenicity

was calculated using grey scale analysis using Image J software (Welch et al., 2020, Wilson et al., 2019). BIA was performed using a Bodystat Quadscan 4000 device. Skeletal Muscle Mass (SMM) was estimated according to the Sergi (Sergi et al., 2015) and Janssen (Janssen et al., 2000) equations. Phase angle was extracted directly from the device. Short Physical Performance Battery (SPPB) (Guralnik et al., 1994) was measured at 13 weeks in the emergency surgery group, at baseline and at 13 weeks in the elective surgery group, and at all timepoints in the medical group. Gait speed alone was measured at 7 days in the surgical groups.

5.2.3.3 Sarcopenia definition

Sarcopenia was defined as low handgrip strength (<16kg in females, <27kg in males), with low BATT (<3.85cm in females, <5.44cm in males) and/or low SMM (<15kg in females, <20kg in males). The presence of sarcopenia was defined at each timepoint.

5.2.3.4 Other clinical information

Demographic data, smoking status, binary coded individual long-term conditions, and binary coded treatments given were collected from the participant and/or patient records. Nutrition was assessed using the Mini-Nutritional Assessment (MNA) at baseline and at 13 weeks. In-hospital step count was recorded using Fitbit Inspire devices (Fitbit, Inc., Google LLC, USA). Delirium was recorded as assessed by the investigating geriatrician at each timepoint, according to the Diagnostic and Statistical Model of Diseases 5 (DSM-5) (American Psychiatric

Association, 2013), or if a diagnosis of delirium was made by the patient's own clinicians at any time during admission. Other variables recorded included length of hospital stay, and hospital readmission with total time spent in hospital.

5.2.3.5 Measurement of systemic biomarkers

Selected biomarkers performed as part of routine clinical care were recorded at each timepoint where available (haemoglobin, creatinine, estimated Glomerular Filtration Rate – eGFR, C-Reactive Protein – CRP, Albumin, white cell count, neutrophil count, and lymphocyte count). Additional blood samples were obtained within 48 hours of admission or surgery in all groups, and preoperatively in the elective surgery group. Plasma cortisol concentration was measured using Human Cortisol ELISA Kit (E-EL-0157, Elabscience), plasma Dehydroepiandrosterone sulfate (DHEA-s) concentration was measured using Human DHEA-s ELISA Kit (EH2946, FineTest, Wuhan Fine Biotech Co., Ltd.), serum High sensitivity CRP (hsCRP) concentration was measured using Human hsCRP ELISA Kit (HK369, HycultBiotech), serum Growth Hormone (GH) concentration was measured using Human Growth Hormone sandwich ELISA kit (KE00167, Proteintech), serum Insulin-like Growth Factor 1 (IGF-1) was measured using Human IGF-1 ELISA Kit (ELH-IGF1, RayBiotech), serum myostatin concentration was measured using Human Myostatin ELISA Kit (DL-MSTN-Hu, Dldevelop), and plasma total 25-hydroxy Vitamin D was measured using Total 25-OH Vitamin D ELISA Kit (80987, Crystal Chem). Serum concentration levels of CCL2/JE/MCP-1, CXCL1/GRO alpha/KC/CINC-1, Flt-2 Ligand/FLT3L, IL-1 alpha/IL-1F1, IL-4, IL-7, IL-10, TNF-alpha, CCL3/MIP-1 alpha, CXCL10/IP-10/CRG-2, IFN-gamma, IL-1 beta/IL-1F2, IL-6, IL-8/CXCL8, IL-15, and VEGF

were measured using Human XL Cytokine Premixed Luminex Performance Assay Kit (1621325, R&D systems, Bio-techne). Resistin and leptin were measured using Human Obesity Premixed Magnetic Luminex Performance Assay Kit (P205396, R&D systems, Bio-techne). Full methodology is included in Appendix 8.6.1.

5.2.3.6 Statistical analysis

5.2.3.6.1 Data description

A full list of variables initially included within the analysis is available in Appendix 8.6.8. This study represents a substudy of the original study; the study was not initially powered for analysis of systemic biomarkers. The original sample size calculation was derived in order to detect clinically significant change in muscle quantity and physical function variables within groups. Due to the COVID-19 pandemic, the sample size calculation was revised in order to enable detection of differences across groups. Baseline characteristics are displayed in text and tables, separated by patient group. Significance of differences were analysed using one-way Analysis of Variance (ANOVA), Chi-squared, Kruskal-Wallis, and Wilcoxon Rank Sum tests. Mean and median values of systemic biomarkers are displayed in table format. Statistical significance of differences between participants with and without sarcopenia at baseline and at 7 days were analysed using unpaired t-tests and Wilcoxon Rank Sum tests as applicable. A heatmap showing all missing values is shown in Figure 8.6-26, Appendix 8.6.8.

5.2.3.6.2 Modelling

Least Absolute Shrinkage and Selection Operator (LASSO) modelling is a penalised regression model able to shrink covariate coefficients towards zero, allowing for the generation of sparse models and concurrently performing feature selection (Tibshirani, 1996) . In this study, LASSO has been applied for both classification and regression to consider prediction of categorical and numerical variables respectively. Firstly, LASSO was applied on data from each timepoint with “SarcAny” as the outcome for classification analysis adjusting for baseline sarcopenia status. Then, information on "Echo", "BATT", and "SMMSergi" at each time point were predicted through regression and their changes from baseline to 7 days, as well as baseline to 13 weeks. In each of these models, data collected at previous timepoints were used to predict future outcomes and due to small sample size of systemic biomarker data, two different analyses were performed: 1) including all data, deleting all features with 30% or more of missing values and imputing those remaining with the median (numerical) or mode (categorical) or, 2) focusing on participants who had systemic biomarker data specifically and imputing any missing values with the median (numerical) or mode (categorical). In total, 64 different models were built, studying the four mentioned outcomes (“SarcAny”, "Echo", "BATT", and "SMMSergi") at each specific timepoint, using all different timepoint data. Moreover, each of those 64 models was bootstrapped from 20 to 70 times, depending on their sample sizes. The number of times features were "selected " in each of the models was counted, and those above the threshold (mean between maximum selected feature and third quartile) had their coefficients averaged and confidence intervals calculated (Chen et al., 2020). More information on data sample sizes and coefficient selection are shown in supplementary tables S3 to S6. Networks were created through igraph (Csardi and Nepusz,

2006) and Cytoscape (Shannon et al., 2003) by combining all of the selected features and outcomes for each data point and using the averaged coefficients as weights. The full code is available at: https://github.com/InFlamUOB/Sarcopenia.

5.2.4 Results

Seventy-nine participants were recruited to the study and included within this analysis. Recruitment and drop-out rates are available in the supplementary data (Figure S1). Full feasibility analysis including screening and recruitment rates has been published previously (Welch et al., 2021). The mean age of participants was 79.1 (6.6) and 39.2% (31/79) were female. Baseline characteristics of participants are shown in Table 1. Blood samples were collected for research purposes for all elective participants (24/24), and within 48 hours of admission/surgery for 64.6% (51/79).

Table 5.2-1 – Baseline characteristics for participants separated by patient cohort.

		Overall (N=79)	Elective surgery (N=24)	Emergency surgery (N=14)	Medical (N=41)	p value
Age – mean (SD)		79.1 (6.6)	76.4 (5.3)	75.2 (4.2)	82.1 (6.7)	<0.001[a]
Gender – Females % (N)		39.2 (31)	50.0 (12)	35.7 (5)	34.1 (14)	0.431[b]
Ethnicity – % (N)	White British	93.7 (74)	95.8 (23)	100 (14)	90.2 (37)	0.742[b]
	White Irish	2.5 (2)	0 (0)	0 (0)	4.9 (2)	
	Indian	2.5 (2)	4.2 (1)	0 (0)	2.4 (1)	
	Arab	1.3 (1)	0 (0)	0 (0)	2.4 (1)	
Body Mass Index (kg/m^2) – mean (SD)		26.5 (6.5)	26.4 (4.3)	24.3 (4.3)	27.4 (8.0)	0.303[a]
Nutritional status – % (N)	Normal	41.8 (33)	75.0 (18)	35.7 (0)	24.4 (10)	0.001[b]
	At risk	50.6 (40)	25.0 (6)	64.3 (9)	61.0 (25)	
	Malnourished	7.6 (6)	0 (0)	0 (0)	14.6 (6)	

[a]One-way ANOVA; [b]Chi-squared test; [c]Kruskal-Wallis test; [d]Wilcoxon Rank Sum test

5.2.4.1 Clinical features

In analysis without systemic biomarkers, clinical features that were shown to be positively associated with sarcopenia status at 7 days (adjusting for baseline sarcopenia status) were anxiety/ depression (preoperative β 0.44, CI 0.17 – 0.72), asthma (48 hours β 0.77, CI 0.61 – 0.92), Chronic Obstructive Pulmonary Disease (COPD) across all timepoints (48 hours β 0.67, CI 0.59 – 0.75), Ischaemic Heart Disease (7 days β 0.7, CI 0.55 – 0.85), and prescription of steroids during admission (48 hours β 1.11, CI 0.98 – 1.24) (Table S8.6-3). White British ethnicity was negatively associated with sarcopenia status at 13 weeks (13 weeks β -0.24, CI -0.32 – -0.15). Clinical features included within sarcopenia diagnosis were negatively associated with sarcopenia status at each timepoint (BATT, SMMSergi, and handgrip strength). Similar patterns were demonstrated in analysis including only participants with systemic biomarkers available (Table S8.6-4).

The presence of delirium was negatively associated with change in BATT to 7 days (7 days β -0.47, CI -0.5 – -0.44), in analysis not including systemic biomarkers (Table S8.6-5). COPD was positively associated with change in BATT to 7 days (7 days β 0.23, CI 0.21 – 0.25). Ischaemic Heart Disease (48 hours β -0.38, CI -0.47 – -0.29) and prescription of metformin were negatively associated with change in SMMSergi to 7 days (48 hours β -0.54, CI -0.67 – -0.41). Diabetes Mellitus was positively associated with change in SMMSergi to 7 days (48 hours β 0.48, CI 0.38 – 0.57). These associations were not replicated in analysis including systemic biomarkers, although COPD (Preoperative β 0.35, CI 0.12 – 0.58), delirium (48 hours β 0.13,

CI 0.06 – 0.2), and metformin prescription (Preoperative β 0.21, CI 0.13 – 0.28) were positively associated with change in echogenicity to 7 days (Table 8.6-6).

5.2.4.2 Systemic biomarkers

Table 5.2-2 shows mean/median concentration levels of systemic biomarkers separated according to sarcopenia status at baseline and at 7 days. Preoperative biomarkers in the elective cohort are presented separately to biomarkers measured within 48 hours of admission/surgery. There were few statistically significant differences between participants with and without sarcopenia in this unadjusted analysis, although some differences appear clinically significant (e.g. lower GH concentration levels in participants with sarcopenia at all timepoints). Participants who met criteria for sarcopenia at baseline had significantly higher IL-7 concentrations levels measured during the acute phase of illness (median 8.78pg/mL vs 6.52pg/mL; p=0.014).

Table 5.2-2 – Mean and median concentrat*ion* levels of systemic biomarkers separated by sarcopenia status

		Baseline		p value	7 days		p value
		No sarcopenia	Sarcopenia		No sarcopenia	Sarcopenia	
Hb (g/L) *Mean*	Preop	120.9 (7.0)	126.5 (7.0)	0.605[a]	123.5 (28.3)	121.3 (22.0)	0.849[a]
	Acute	113.7 (21.9)	114.5 (16.9)	0.850[a]	113.2 (18.4)	110.5 (17.2)	0.561[a]
WCC (10^9/L) *Median*	Preop	7.0 (6.1 – 8.4)	7.3 (6.15 – 8.5)	0.987[b]	6.85 (6.45 – 8.2)	7.7 (6.15 – 9.65)	0.778[b]
	Acute	10.6 (7.7 – 13.9)	9.5 (8.1 – 13.4)	0.976[b]	9.45 (8.05 – 13.3)	10.85 (8.2 – 14.3)	0.568[b]
Neutrophils (10^9/L)	Preop	4.75 (4.3 – 6.1)	4.7 (3.45 – 6.15)	0.801[b]	4.45 (3.9 – 5.65)	4.95 (3.45 – 6.85)	0.779[b]

Median	Acute	7.9 (6.1 – 11.7)	7.9 (6.0 – 12.2)	0.976[b]	7.4 (6.15 – 11.4)	8.65 (5.8 – 12.3)	0.821[b]
Lymphocytes (10^9/L) *Median*	Preop	1.3 (0.9 – 2.0)	1.45 (1.25 – 1.75)	0.709[b]	1.5 (1.2 – 2.5)	1.45 (1.2 – 1.9)	0.820[b]
	Acute	0.7 (0.5 – 1.1)	0.8 (0.5 – 1.3)	0.229[b]	0.7 (0.5 – 1.1)	0.8 (0.6 – 1.3)	0.224[b]
Albumin (g/L) *Mean*	Preop	38.6 (3.4)	35.9 (1.3)	0.096[a]	39.3 (3.9)	37.1 (3.4)	0.214[a]
	Acute	29.6 (5.6)	28.8 (5.4)	0.537[a]	30.1 (5.7)	29.2 (5.5)	0.509[a]
Creatinine (micromol/L) *Median*	Preop	89 (81 – 101)	74 (67 – 85)	0.170[b]	89 (77.5 – 89.5)	82.5 (69 – 102)	0.836[b]
	Acute	92 (72 – 120)	81.5 (61 – 111)	0.180[b]	87 (78 – 132)	84.5 (61 – 111)	0.275[b]
eGFR *Median*	Preop	76 (41 – 81)	67 (60 – 89.5)	0.731[b]	71 (53 – 76)	65 (53 – 90)	0.678[b]
	Acute	60 (46 – 81)	68.5 (48 – 90)	0.409[b]	60 (46 – 81)	71 (47 – 90)	0.545[b]
hsCRP (mg/L) *Median*	Preop	8.17 (5.74 – 9.18)	4.72 (1.01 – 8.07)	0.170[b]	7.01 (1.92 – 9.36)	7.82 (1.63 – 9.00)	0.902[b]
CRP (mg/L) *Median*	Acute	99 (75 – 158)	113 (78 – 194)	0.272[b]	123 (90.5 – 174.5)	109 (67 – 194)	0.568[b]
Myostatin (ng/mL) *Mean*	Preop	20.8 (15.5)	22.7 (9.9)	0.754[a]	15.1 (10.1)	26.1 (14.1)	0.063[a]
	Acute	26.2 (20.2)	29.1 (3.0)	0.565[a]	23.7 (18.1)	28.6 (15.4)	0.332[a]
Cortisol (ng/mL) *Median*	Preop	57.5 (35.4 – 75.3)	100.9 (44.5 – 190.5)	0.238[b]	68.9 (36.5 – 74.9)	73.0 (38.5 – 167.8)	0.345[b]
	Acute	85.1 (75.0 – 123.3)	78.9 (78.0 – 104.8)	0.915[b]	78.5 (90.5 – 115.3)	90.7 (67.0 – 111.6)	0.643[b]
DHEA-s (ng/mL) *Median*	Preop	192.2 (176.9 – 311.1)	160.9 (150.6 – 230.1)	0.192[b]	192.9 (178.5 – 288.7)	193.1 (150.6 – 357.4)	0.862[b]
	Acute	291.7 (159.7 – 426.0)	219.7 (95.3 – 503.0)	0.307[b]	255.6 (159.7 – 392.2)	219.7 (95.3 – 581.5)	0.831[b]
IGF-1 (ng/mL) *Median*	Preop	2.92 (1.72 – 5.60)	2.22 (0.84 – 4.65)	0.566[b]	1.51 (0.62 – 4.65)	2.75 (2.24 – 3.62)	0.607[b]
	Acute	1.83 (0.78 – 8.58)	2.87 (0.37 – 12.2)	0.825[b]	1.56 (0.69 – 18.3)	2.41 (0.37 – 6.57)	0.771[b]
Growth Hormone (pg/mL) *Median*	Preop	406.5 (118.9 – 1643.8)	234.2 (81.4 – 491.7)	0.443[b]	1087.1 (217.8 – 2116.0)	164.9 (102.2 – 491.7)	0.122[b]
	Acute	1327.1 (478.4 – 2375.1)	812.8 (514.3 – 2076.5)	0.492[b]	1284.3 (478.4 – 3231.8)	985.4 (514.3 – 1463.0)	0.545[b]
Vitamin D (ng/mL) *Median*	Preop	19.3 (6.6 – 23.7)	20.7 (15.4 – 24.8)	0.662[b]	20.9 (1.8 – 23.2)	18.3 (15.4 – 24.8)	0.371[b]
	Acute	14.4 (3.5 – 22.9)	19.1 (3.1 – 24.0)	0.602[b]	13.4 (3.2 – 22.1)	19.3 (4.6 – 24.1)	0.520[b]

CCL2 (pg/mL) *Median*	Preop	223.6 (186.4 – 289.2)	346.1 (188.3 – 540.5)	0.166[b]	228.4 (186.4 – 289.2)	309.3 (183.8 – 520.2)	0.370[b]
	Acute	252.9 (157.2 – 445.3)	291.8 (159.9 – 355.6)	0.931[b]	226.5 (149.3 – 355.6)	303.8 (166.8 – 421.2)	0.217[b]
CXCL10 (pg/mL) *Median*	Preop	5.08 (2.22 – 53.3)	51.6 (17.9 – 125.2)	0.093[b]	24.1 (6.1 – 65.4)	42.2 (10.1 – 112.1)	0.755[b]
	Acute	2.4 (2.2 – 39.4)	12.6 (4.6 – 59.5)	0.242[b]	10.6 (2.2 – 42.3)	8.4 (2.2 – 59.5)	0.929[b]
IL-1a (pg/mL) *Median*	Preop	12.2 (10.7 – 12.2)	13.7 (12.2 – 15.8)	0.154[b]	12.2 (12.2 – 12.2)	13.7 (10.7 – 13.7)	0.719[b]
	Acute	10.7 (9.2 – 13.7)	12.6 (10.7 – 14.4)	0.176[b]	10.7 (9.2 – 13.7)	12.2 (10.7 - 13.7)	0.720[b]
IL-6 (pg/mL) *Median*	Preop	9.4 (7.0 – 10.4)	7.5 (4.0 – 103.4)	0.203[b]	8.4 (7.0 – 10.4)	8.9 (5.1 – 12.4)	0.952[b]
	Acute	88.8 (21.4 – 155.8)	37.6 (23.0 – 103.4)	0.170[b]	69.2 (27.8 – 130.6)	37.6 (25.2 – 106.0)	0.397[b]
IL-10 (pg/mL) *Median*	Preop	29.9 (16.0 – 58.7)	39.4 (23.1 – 301.9)	0.598[b]	44.4 (16.1 – 107.4)	37.0 (27.5 – 51.4)	0.976[b]
	Acute	32.3 (22.9 – 52.4)	66.0 (28.7 – 194.1)	0.094[b]	51.4 (22.9 – 80.8)	41.7 (25.2 – 80.8)	0.970[b]
VEGF (pg/mL) *Median*	Preop	109.5 (80.6 – 163.4)	120.6 (78.8 – 190.0)	0.973[b]	101.0 (80.6 – 155.6)	140.1 (83.2 – 209.3)	0.370[b]
	Acute	262.2 (126.0 – 382.1)	273.2 (158.9 – 459.5)	0.561[b]	270.3 (132.1 – 349.5)	239.2 (137.9 – 486.4)	0.857[b]
IL-7 (pg/mL) *Median*	Preop	6.32 (4.93 – 6.93)	7.44 (5.52 – 8.16)	0.132[b]	6.52 (6.12 – 6.93)	6.93 (5.32 – 8.57)	0.399[b]
	Acute	6.52 (5.72 – 7.75)	8.78 (6.52 – 10.25)	0.014*[b]	6.52 (6.12 – 7.75)	8.42 (6.32 – 10.78)	0.063[b]
IL-15 (pg/mL) *Median*	Preop	3.33 (2.66 – 3.56)	3.33 (3.22 – 3.45)	0.722[b]	3.33 (3.33 – 3.79)	3.33 (2.88 – 5.03)	0.392[b]
	Acute	3.78 (3.1 – 4.5)	4.25 (3.33 – 5.0)	0.133[b]	3.79 (3.1 – 4.25)	4.14 (3.33 – 5.03)	0.278[b]
CXCL1 (pg/mL) *Median*	Preop	69.4 (61.7 – 101.3)	76.2 (35.4 – 145.1)	0.829[b]	67.9 (61.7 – 99.0)	84.5 (47.6 – 139.0)	0.515[b]
	Acute	97.8 (58.1 – 157.8)	110.0 (70.9 – 161.1)	0.465[b]	98.4 (64.9 – 138.4)	103.3 (65.22 – 148.8)	0.713[b]
IL-1b (pg/mL) *Median*	Preop	2.04 (1.29 – 3.52)	2.23 (2.04 – 3.15)	0.477[b]	2.04 (1.29 – 2.04)	2.41 (2.04 – 3.52)	0.050*[b]
	Acute	2.04 (1.29 – 2.78)	2.04 (1.67 – 2.78)	0.461[b]	1.67 (1.29 – 2.78)	2.04 (2.035 – 2.78)	0.142[b]
IL-8 (pg/mL) *Median*	Preop	11.0 (6.7 – 16.3)	21.7 (10.5 – 30.4)	0.110[b]	12.1 (10.0 – 17.8)	16.3 (9.0 – 29.4)	0.656[b]
	Acute	11.2 (6.3 – 23.2)	13.2 (10.5 – 21.3)	0.280[b]	10.2 (6.3 – 18.0)	13.2 (10.3 – 29.3)	0.144[b]

TNF-α (pg/mL) *Median*	Preop	11.2 (8.8 – 14.8)	16.0 (8.8 – 22.7)	0.254[b]	11.6 (8.8 – 17.3)	15.0 (8.8 – 17.7)	0.719[b]
	Acute	14.8 (10.0 – 16.9)	13.6 (11.2 – 18.5)	0.668[b]	15.2 (10.4 – 18.5)	12.8 (10.8 – 18.1)	0.765[b]
Leptin (pg/mL) *Median*	Preop	13914 (9619 – 22260)	12328 (6340 – 32851)	0.881[b]	16368 (9940 – 17482)	16979 (9292 – 26582)	0.719[b]
	Acute	13871 (5510 – 32766)	4598 (2109 – 17928)	0.668[b]	14262 (5716 – 28725)	5711 (2251 – 21709)	0.765[b]
Resistin (pg/mL) *Median*	Preop	8873 (7130 – 13602)	9616 (7725 – 13536)	0.788[b]	7949 (7068 – 13941)	9952 (8468 – 12564)	0.719[b]
	Acute	20625 (11828 – 28384)	17165 (11356 – 27014)	0.668[b]	20970 (13144 – 41934)	15394 (11167 – 23891)	0.765[b]

[a]Unpaired t-test; [b]Wilcoxon rank-sum test

Hb=Haemoglobin; WCC=White Cell Count; eGFR=estimated Glomerular Filtration Rate; hsCRP=High sensitivity C-Reactive Protein; CRP=C-Reactive Protein; DHEA-s=Dehydroepiandrosterone sulfate; IGF-1=Insulin-like Growth Factor 1; CCL2=Chemokine (C-C motif) ligand 2; CXCL10=Chemokine (C-X-C motif) ligand 10; IL-1a=Interleukin 1a; IL-6=Interleukin 6; IL-10=Interleukin 10; VEGF=Vascular Endothelial Growth Factor; IL-7=Interleukin 7; IL-15; Interleukin 15; CXCL1=Chemokine (C-X-C motif) ligand 1; IL-1b=Interleukin 1b; IL-8=Interleukin 8; TNF-α=Tumour Necrosis Factor Alpha

IL-1b measured within 48 hours of admission/surgery was positively associated with sarcopenia status at 7 days (β 0.24, CI 0.06 – 0.42), and resistin was negatively associated (β -0.12, CI -0.23 – -0.01). TNFα measured both preoperatively and within 48 hours of admission/surgery was negatively associated with change in echogenicity and positively associated with change in SMMSergi to 7 days. Serum creatinine was positively associated with change in SMMSergi to 7 days; eGFR was negatively associated with change in BATT to 7 days.

5.2.4.3 Network analyses

Figure 5.2-1 shows the network generated for outcomes including systemic biomarkers measured preoperatively. Preoperative IL7 was positively associated with echogenicity preoperatively and at 7 days. Preoperative TNFα was positively associated BATT and SMMSergi at 7 days. Variables associated with echogenicity appeared to cluster separately from variables associated with measures of muscle quantity.

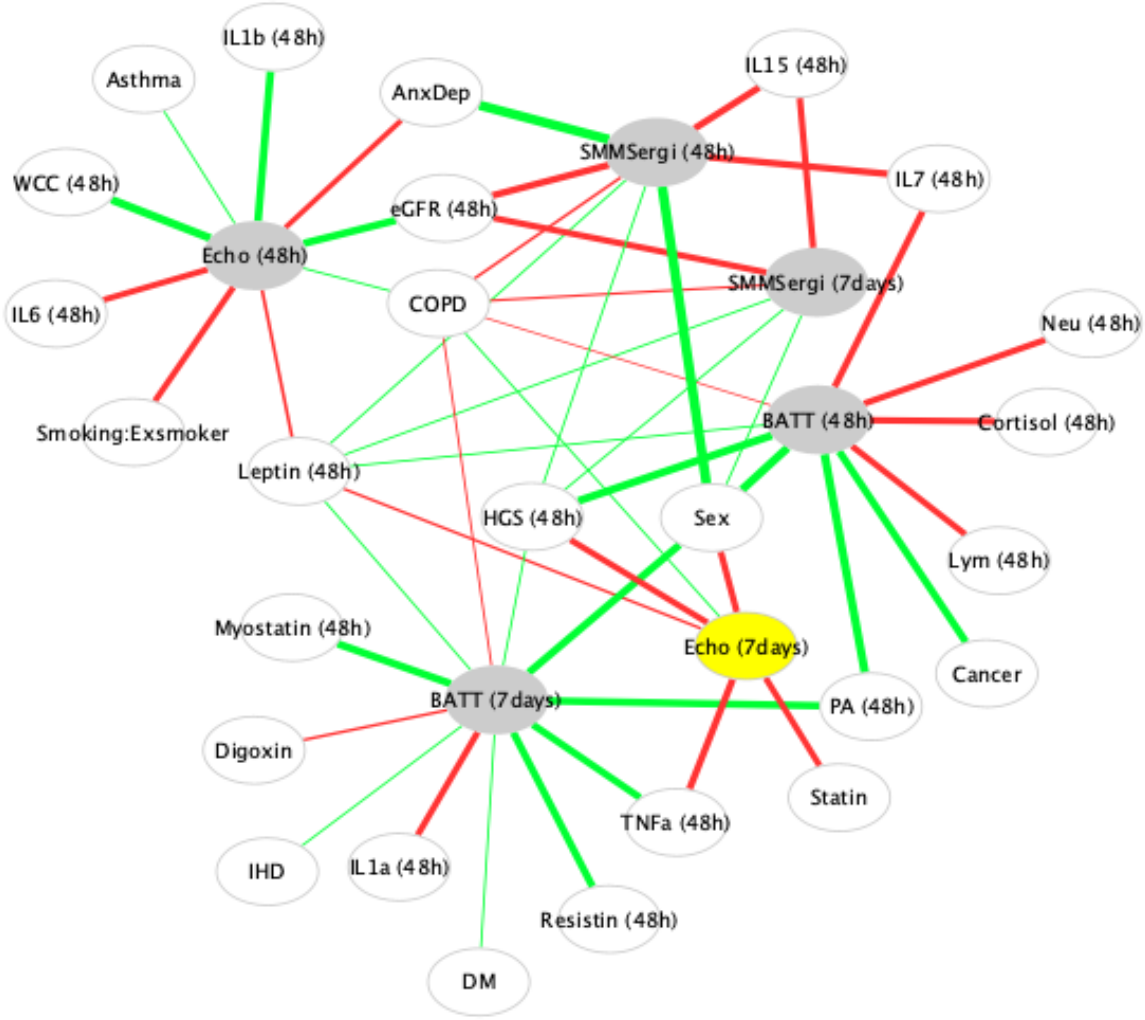

Figure 5.2-2 shows the network generated for outcomes including systemic biomarkers measured within 48 hours of surgery/admission. In this network, COPD showed consistent

positive associations with echogenicity and negative associations with measures of muscle quantity.

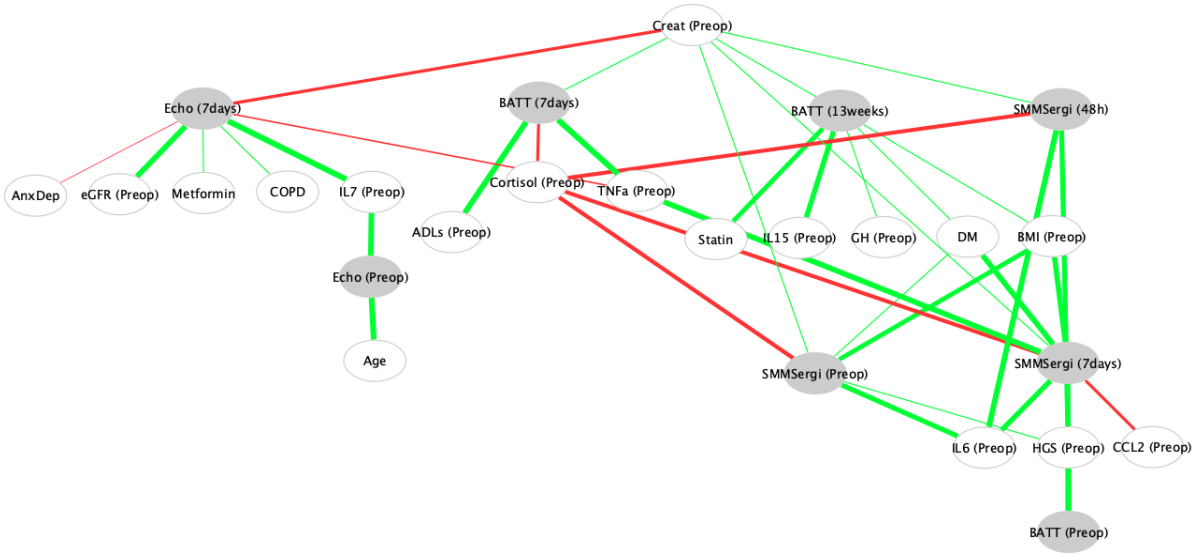

Figure 5.2-1 – Network derived from continuous variable outcomes including systemic biomarkers measured preoperatively.

Red lines show negative associations and green lines show positive associations. *Echo=Echogenicity; BATT=Bilateral Anterior Thigh Thickness; SMMSergi=Skeletal Muscle Mass (Sergi equation); AnxDep=Anxiety/Depression; eGFR=estimated Glomerular Filtration Rate; COPD=Chronic Obstructive Pulmonary Disease; IL7=Interleukin 7; ADLs=Activities of Daily Living; TNFa=Tumour Necrosis Factor Alpha; IL15=Interleukin 15; GH=Growth Hormone; DM=Diabetes Mellitus; BMI=Body Mass Index; IL6=Interleukin 6; HGS=Handgrip Strength; CCL2=Chemokine (C-C motif) ligand 2*

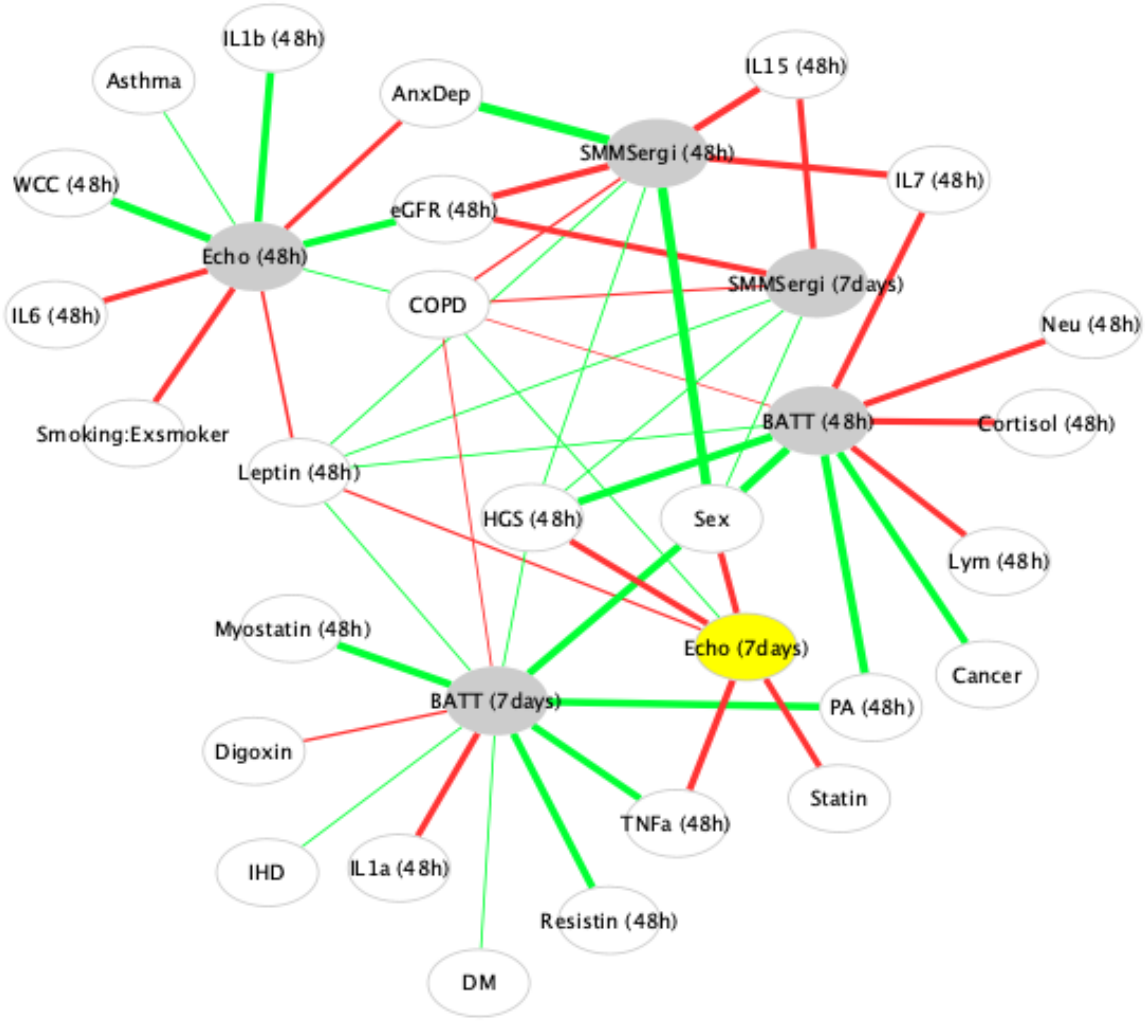

Figure 5.2-2 – Network derived from continuous variable outcomes including systemic biomarkers measured within 48 hours of surgery/admission.

Red lines show negative associations and green lines show positive associations. *IL6=Interleukin 6; WCC=White Cell Count; IL1b=Interleukin 1 beta; AnxDep=Anxiety/Depression; Echo=Echogenicity; eGFR=estimated Glomerular Filtration Rate; COPD=Chronic Obstructive Pulmonary Disease; SMMSergi=Skeletal Muscle Mass (Sergi equation); IL15=Interleukin 15; IL7=Interleukin 7; BATT=Bilateral Anterior Thigh Thickness; Neu=Neutrophil count; HGS=Handgrip strength; Lym=Lymphocyte count; TNFa=Tumour Necrosis Factor Alpha; IL1a=Interleukin 1 Alpha; IHD=Ischaemic Heart Disease*

5.2.5 Discussion

These results provide proof-of-concept towards the identification of clinical features and systemic biomarkers related to sarcopenia in hospitalised older patients, which will guide

future research to enable clinical risk stratification and novel intervention strategies. COPD was consistently positively associated with sarcopenia status at 7 days in association with clinical features measured at all timepoints, in a high proportion of models. This association was demonstrated despite adjusting for baseline sarcopenia status, suggesting that this association may be distinct from any association with chronic sarcopenia. Conversely, COPD was positively associated with change in BATT, although it was positively associated with change in echogenicity i.e., increased muscle quantity but reduced muscle quality. Whilst echogenicity did not form part of the sarcopenia diagnosis used in this study, it is recognised that reduced muscle quality (i.e., elevated echogenicity) may be important to pathogenesis, and can be used in place of reduce muscle quantity in sarcopenia diagnosis (Cruz-Jentoft et al., 2019). Prescription of steroids at any point during admission was positively associated with sarcopenia at 7 days. This effect was also demonstrated consistently alongside clinical features measured at all timepoints and in high proportions of models. Steroid treatment has been shown to exacerbate loss of muscle quantity during bedrest in healthy adults (Paddon-Jones et al., 2006) and upregulate pathways of muscle protein degradation in rodent models (Bodine et al., 2001). Patients with COPD are more likely to have been prescribed steroids acutely during admission as part of treatment for acute exacerbations, as well as to have received steroids previously, but there may also be separate innate common pathways within COPD aetiology.

Prescription of metformin was negatively associated with change in SMMSergi (in analysis without cytokines, in combination with clinical features at 48 hours) and positively associated with change in echogenicity (in analysis with cytokines, in combination with preoperative

clinical features) to 7 days. This suggests that prescription of metformin may negatively impact on muscle quantity and quality. However, these effects were not consistent and there was no clear association with sarcopenia itself. Diabetes Mellitus was positively associated with change in SMMSergi and negatively associated with change in echogenicity, suggesting that the effects of metformin are distinct from any effect from Diabetes Mellitus. Metformin reduces inflammation and in rodent models has been shown to reduce fat infiltration within muscles following thermal injury (Yousuf et al., 2020). On the other hand, evidence suggests that it may actually promote muscle protein breakdown and reduce muscle protein synthesis (Walton et al., 2019). Studies are currently ongoing into the role of metformin in the treatment and prevention of chronic sarcopenia.

In our study there was a negative association with White British ethnicity and sarcopenia at 13 weeks. This suggests that patients who self-identify with other ethnic backgrounds may be at increased risk of poor recovery of muscle quantity and function following hospitalisation. The majority of participants recruited to this study were from a White British background, and we did not measure socioeconomic status as part of this study, which could account for these differences. However, this effect requires urgent further evaluation. Previous studies have demonstrated that older people who self-identify as belonging to a minority ethnic group have lower health-related quality of life compared to those who identify as White British (Watkinson et al., 2021).

Higher serum concentrations of IL-7 were measured during the acute phase of illness in participants who met criteria for sarcopenia at baseline. IL-7 is expressed and secreted by human skeletal muscle cells (Haugen et al., 2010). Whilst this process may be physiological, excessive secretion may lead to increased systemic inflammation and immune dysregulation. This suggests that chronic sarcopenia may be associated with dysregulated myokine secretion and immune adaptations. Sarcopenia has been consistently shown to be associated with increased risk of mortality and adverse outcomes (Beaudart et al., 2017), even when adjusting for factors such as comorbidities and functional status (Vetrano et al., 2014). It is possible that these associations may relate to immune dysregulation directly precipitated by dysregulated muscle secretory processes in sarcopenia. Median GH concentrations were increased in the acute phase compared to preoperative levels in participants both with and without sarcopenia. However, concentrations remained consistently lower in participants with sarcopenia. This suggests that reduced baseline GH may lead to an ineffective surge with acute illness, and reduced promotion of muscle synthesis. GH is known to decline with age (Junnila et al., 2013). GH l may increase with acute illness but with a state of peripheral GH resistance accompanied by low IGF-1 levels (Ross et al., 1991). GH has been shown to promote muscle protein synthesis in healthy volunteers (Fryburg and Barrett, 1993).

The presence of delirium was negatively associated with change in BATT and positively associated with change in echogenicity to 7 days (reduced muscle quantity and quality). These results are novel and merit further evaluation. Previous studies have shown that low baseline skeletal muscle mass is a risk factor for incident delirium (Mosk et al., 2018), and that delirium is independently associated with risk of being sarcopenic upon admission to a geriatric unit

(Bellelli et al., 2018). However, we are not aware of previous studies that have assessed the association of changes in muscle quantity and quality with the presence of delirium. Delirium is considered to relate to processes of systemic inflammation and immune dysregulation (Kealy et al., 2020); these changes in turn may lead to increased risk of muscle protein breakdown. Additionally, delirium has been shown to be associated with reduced physical activity and prolonged bedrest during hospitalisation (Fisher et al., 2011), which is known to be associated with increased risk of muscle wasting in older adults (Kortebein et al., 2007) .

No systemic biomarkers were clearly and consistently associated with sarcopenia status at 7 days or change in BATT, SMMSergi, or echogenicity. However, there was a positive association between IL-1b serum concentrations and sarcopenia status at 7 days. IL-1b is a pro-inflammatory cytokine secreted with acute inflammation. IL-1b has been shown to be expressed in myocytes in rodent models of sepsis and is considered to be a key mediator of muscle atrophy in this context (Huang et al., 2017). Interestingly, serum and cerebrospinal fluid concentration levels of IL-1b are elevated in patients with delirium (Cape et al., 2014), which may explain the association demonstrated in this study between delirium and sarcopenia in the acute setting.

Interestingly, eGFR was shown to be negatively associated with change in BATT, and positively associated with change in echogenicity to 7 days. This would suggest that participants with better renal function had reduced muscle quantity and quality. However, creatinine was positively associated change in SMMSergi, and negatively associated with change in

echogenicity at 7 days. Creatinine is a known biomarker of muscle quantity, as well as renal function, and the eGFR is derived from creatinine by the Modification of Diet in Renal Disease study equation (Levey et al., 2006). It is counter-intuitive that improved renal function would be associated with reduced muscle quantity and quality, and it is more biologically plausible that this association relates to reduced serum creatinine levels with low muscle quantity. However, this suggests that the eGFR may be less reliable as a measure of renal function in patients with reduced skeletal muscle mass. The Cockcroft-Gault formula, which also considers the patient's weight may be a more suitable alternative formula for estimation of renal function in older people at risk of sarcopenia (Cockcroft and Gault, 1976).

5.2.5.1 Strengths and limitations

This study presents results derived from clinical and laboratory-based research. All clinical assessments were completed by a clinician with training and experience in geriatric medicine. Statistical analysis was performed independently by a bioinformatician who was not involved in the collection of data for this study; robust methods were applied through the use of bootstrapping in model building. Demonstration of association of clinical features (BATT, SMMSergi, handgrip strength) used in the diagnosis of sarcopenia with sarcopenia status supports reliability of the models. However, it is important to note that the study was underpowered compared to the original planned sample size calculation, which was revised in light of the COVID-19 pandemic. The exclusion of variables with greater than 30% missing values ensured robustness of the models, but may have also led to exclusion of some variables that may have been of significance. Additionally, due to high numbers of participants where

data were collected remotely at 13 weeks, many variables and outcomes were excluded from analysis at 13 weeks. Therefore, the results predominantly focus on biomarkers in relation to sarcopenia status at 7 days. The purpose of this analysis was to demonstrate associations towards proof-of-concept to guide future mechanistic, observational, and interventional studies. We, therefore, have not commented on the size or magnitude of significance of associations, which is the common approach for all network analyses. Simple unadjusted analyses were performed when comparing mean/median biomarker concentrations shown in Table 2. These data were predominantly presented for descriptive purposes, but it is important to note that these differences do not account for differences between sex and patient groups.

5.2.5.2 Recommendations for future research

Further mechanistic studies should aim to further assess inflammatory pathways involved in muscle atrophy in the acute setting. The role of IL-1b should be explored further and may potentially serve as a biomarker in risk stratification. The results of this study did not clearly demonstrate potential interventions, but results may be used for comparison when designing and conducting trials including theoretical interventions related to the biomarkers measured in this study (e.g. GH injection, myostatin inhibitors). In considering how treatments are targeted to ensure greatest benefit, initially targeting treatment towards patients on treatment with steroid medication would be a pragmatic approach. This would include patients on treatment with prednisolone for exacerbations of COPD or asthma, as well as patients on treatment with dexamethasone for symptomatic COVID-19 infection. Patients

with delirium are likely to be another group who would particularly benefit from targeted interventions, which will need to be carefully tailored to ensure effectiveness and feasibility in clinical practice.

5.2.6 Conclusion

Acute sarcopenia is a complex phenomenon and it is unlikely that a single biomarker would be sensitive or specific enough to identify or predict the onset of acute sarcopenia alone. No systemic biomarkers were consistently associated with both sarcopenia status at 7 days and changes in muscle quantity and quality at 7 days post-admission/surgery, although IL-1b was positively associated with sarcopenia status. Patients that may be considered most at risk include patients with heightened systemic inflammation, who are prescribed steroid medications, or diagnosed with delirium. Further mechanistic studies are warranted to elucidate underlying pathways to guide therapeutic interventions. At the same time, interventional studies should not be delayed and pragmatic studies of interventions with biological plausibility are encouraged.

5.2.7 References

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6 Towards interventions for acute sarcopenia

Chapter 6.1 - Interventions to ameliorate reductions in muscle quantity and function in hospitalised older adults: a systematic review towards acute sarcopenia treatment

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Author contributions: CW devised the protocol and search strategy for this study. CW and ZM completed all manuscript selection against inclusion/exclusion criteria, data extraction, and risk of bias assessment in duplicate. CW synthesised the results and drafted the manuscript. CG, TM, and TAJ provided supervision to CW, agreed the original protocol, and contributed to the manuscript. JG contributed to the interpretation of the results. All authors agreed the final submitted version.

6.1 Interventions to ameliorate reductions in muscle quantity and function in hospitalised older adults: a systematic review towards acute sarcopenia treatment

6.1.1 Abstract

Objective: Assimilate evidence for interventions to ameliorate negative changes in physical performance, muscle strength, and muscle quantity in hospitalised older adults.

Methods: We searched for articles using MEDLINE, Embase, CINAHL, and Cochrane library using terms for randomised controlled trials, older adults, hospitalisation, and change in muscle quantity, strength, or physical performance. Two independent reviewers extracted data and assessed risk of bias. We calculated standardised mean differences for changes in muscle function/quantity pre- and post-intervention.

Results: We identified 9805 articles; 9614 were excluded on title/abstract; 147 full texts were excluded. We included 44 studies including 4522 participants; mean age 79.1. Twenty-seven studies (n=3417) involved physical activity interventions; a variety were trialled. Eleven studies involved nutritional interventions (n=676). One trial involved testosterone (n=39), two involved Growth Hormone (n=53), one involved nandrolone (n=29), and another involved erythropoietin (n=141). Three studies (n=206) tested Neuromuscular Electrical Stimulation. Evidence for effectiveness/efficacy was limited. Strongest evidence was for multi-component physical activity interventions. However, all studies exhibited at least some concerns for overall risk of bias, and considering inconsistencies of effect sizes across studies, certainty around true effect sizes is limited.

Conclusion: There is currently insufficient evidence for effective interventions to ameliorate changes in muscle function/quantity in hospitalised older adults. Multiple interventions have been safely trialled in heterogeneous populations across different settings. Treatment may need to be stratified to individual need. Larger scale studies testing combinations of interventions are warranted. Research aimed at understanding pathophysiology of acute sarcopenia will enable careful risk stratification and targeted interventions.

Registration: The protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) – CRD42018112021.

6.1.2 Introduction

Sarcopenia is defined by low muscle strength with low muscle quantity/quality; additionally demonstrated low physical performance defines severe sarcopenia. Cut-offs are two Standard Deviations (SDs) below means of young healthy reference populations (Cruz-Jentoft et al., 2018a). Acute sarcopenia (acute muscle insufficiency) particularly affects hospitalised older adults (Cruz-Jentoft and Sayer, 2019, Welch et al., 2018). Normally proceeded by stressor events, it is defined by acute declines in muscle quantity/quality and/or function (strength or physical performance) producing incident sarcopenia (Cruz-Jentoft et al., 2018b, Welch et al., 2018). Previous reviews considered chronic sarcopenia treatment/prevention (Yoshimura et al., 2017, Beaudart et al., 2017, Cruz-Jentoft et al., 2014); strongest evidence exists for physical activity. Resistance training improves muscle quantity, strength, and physical performance in community-dwelling populations (Beckwee et al., 2019). Some trials demonstrated enhanced benefit of nutritional supplementation alongside (Denison et al.,

2015). Large studies are underway evaluating combined nutritional and exercise interventions for chronic sarcopenia (Marzetti, 2018).

It is unknown whether chronic sarcopenia interventions can treat acute sarcopenia. Mechanisms differ, which may affect treatment efficacy. Acute sarcopenia is associated with greater systemic inflammation and immune-endocrine dysregulation. Inflammation (acute or chronic) may blunt response to exercise or protein challenges (anabolic resistance), but this may be acutely/severely upregulated in acute sarcopenia (Morton et al., 2018). Acute sarcopenia follows an accelerated course (Welch et al., 2018); traditional treatments may not work fast enough. Additionally, community interventions may be unfeasible in hospital. This review aimed to identify trialled interventions for ameliorating negative changes in muscle quantity, strength, or physical performance in hospitalised older adults, and to summarise/synthesise findings.

6.1.3 Methods

6.1.3.1 Protocol and registration

Protocol was agreed by all researchers and registered with Prospective Register of Systematic Reviews (PROSPERO) – CRD42018112021. Reporting is consistent with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidance.

6.1.3.2 Eligibility criteria

We included randomised controlled trials (RCTs) and quasi-randomised controlled trials involving hospitalised patients ≥65 years-old, where pre- and post-intervention measurements of muscle quantity, strength, or physical performance were available. Post-intervention measures until 28 days post-intervention were included. We included physical activity, nutritional, pharmaceutical, or Neuromuscular Electrical Stimulation (NMES) trials. Exclusion criteria were: degenerative neuromuscular disorders; acute stroke; trials of parenteral nutrition, surgical technique/invasive procedure, chemotherapy/radiotherapy, or anaesthetic agents/techniques; no control group; lengths of stay less than two days. We included studies that measured muscle quantity using Computed Tomography (CT), Magnetic Resonance Imaging (MRI), Dual Energy X-ray Absorptiometry (DXA), Bioelectrical Impedance Analysis (BIA), or ultrasound, muscle strength using handgrip strength, knee flexion, or knee extension, or physical performance using Short Physical Performance Battery (SPPB), gait speed, Timed Up and Go (TUG), or 6-Minute Walking Test (6MWT). There were no date or language restrictions.

6.1.3.3 Information sources

We searched electronic databases (MEDLINE, Embase, CINAHL, CENTRAL) on 16th January 2019; search repeated on 3rd April 2020. Grey literature was identified through Web of Science, Google Scholar, Clinicaltrials.gov, article references, and protocol citations. We contacted authors for information where necessary, including requesting age breakdown of

data. If no response was obtained, a decision was made to include studies where mean age was one SD greater than 65.

6.1.3.4 Search strategy

We used published and unpublished terms for study design (RCTs), population (older adults AND hospitalised) and outcome measures (muscle mass OR muscle strength OR physical performance) in our search. Full search strategy is available in the online supplement (Appendix 8.7.1); this was reviewed and agreed with an information specialist.

6.1.3.5 Study selection

Citations were imported into Microsoft Excel 2016. Duplicates were removed automatically/manually. Two reviewers independently screened titles and abstracts for inclusion (CW, ZM). Disagreements were resolved through discussion. Full texts were reviewed independently by the same reviewers; disagreements were resolved through discussion or third review (TAJ).

6.1.3.6 Data extraction

Data were extracted independently by two reviewers (CW, ZM) using a template (Microsoft Excel 2016). Extracted data were country, study design, sample size and dropouts, sample

characteristics (age, ethnicity, Body Mass Index – BMI, sex), speciality, intervention description (type of intervention, how delivered), intervention characteristics (timing of intervention, dosage), control group, outcome data, length of stay, and adverse events. Outcome data at baseline and follow-up to include muscle quantity, muscle strength, and physical performance were extracted.

6.1.3.7 Risk of bias

Two reviewers (CW, ZM) independently assessed risk of bias using Cochrane risk of bias tool. Conflicts were resolved by discussion. Risk of bias was collated using RevMan version 5.3 (The Cochrane Collaboration, 2014).

6.1.3.8 Synthesis of results

We summarised study and participant characteristics, and outcome data at baseline and follow-up using means/SDs in text and tables. Interventions were grouped by subtype and outcomes. All studies were included in narrative synthesis. If sufficient information was available to estimate Standardised Mean Differences (SMDs) of change scores, effect sizes were evaluated as described in statistical analysis section. Certainty of interventions with large effect sizes was evaluated using Grading of Recommendations, Assessment, Development and Evaluations (GRADE) (Siemieniuk).

6.1.3.9 Statistical analysis

Correlations for outcome measures were calculated from studies reporting SDs of change scores and baseline/follow-up measures (The Cochrane Collaboration, 2011a). Mean correlation for each outcome was used to estimate SD of change in outcomes in studies where this was not available. We calculated SMDs of change scores by dividing difference in change score between comparison and intervention groups by SD of change score in comparison group (The Cochrane Collaboration, 2011b). Effect sizes were calculated to one decimal place and classified as no effect (≤0.1), small (0.2 – 0.4), medium (0.5 – 0.7), or large (0.8 or greater) (Cohen, 1992). If more than one effect size was available for a single trialled intervention and outcome type, the larger was included. Meta-analysis was not performed due to high heterogeneity of interventions and outcomes.

6.1.4 Results

6.1.4.1 Study selection

We identified 9805 articles after duplicates removal. We excluded 9613 following title/abstract screening; 192 full texts assessed for eligibility. We excluded 148 full text articles due to mean age not more than one SD above 65 (n=56), no control group (n=10), follow-up over 28 days (n=12), no baseline measures (n=6), no measures meeting inclusion criteria (n=20), duplicate data (n=11), unable to obtain necessary data from authors (n=24), other

intervention type (n=2), and non-hospitalised population (n=6) (Figure 6.1-2). We included 44 studies in narrative synthesis and 32 studies in effect size evaluation.

6.1.4.2 Study characteristics

This review included 4522 participants (2160 control, 2362 intervention). Sample size per arm ranged from 7 to 232. Most studies were small; 52% (23/44) (Wnuk et al., 2016, Rahmann et al., 2009, Giangregorio et al., 2009, Fiore et al., 2017, Tal-Akabi et al., 2007, Said et al., 2012, Torres-Sanchez et al., 2017, Blanc-Bisson et al., 2008, Henriksen et al., 2002, Niccoli et al., 2017, Hermanky et al., 2017, Saudny-Unterberger et al., 1997, Ogasawara et al., 2018, Bouillanne et al., 2018, Weissberger et al., 2003, Hedström et al., 2004, Sloan et al., 1992, Zinglersen et al., 2018, Martin-Salvador et al., 2016, McGowan et al., 2018, Braun et al., 2019, Deer et al., 2019, Files et al., 2020, Prasciene et al., 2019) included 30 or fewer participants per arm; only 9% (4/44) (de Morton et al., 2007, Raymond et al., 2017, Martínez-Velilla et al., 2019) included over 100 participants in both arms. Mean age across all studies was 79.1 years; 59% female. Of studies reporting BMI, 74% (20/27) (Wnuk et al., 2016, Rahmann et al., 2009, Zinglersen et al., 2018, Busch et al., 2012, McCullagh, 2017, Martínez-Velilla et al., 2019, Tal-Akabi et al., 2007, Houborg et al., 2005, Sano, 2018, Torres-Sanchez et al., 2017, Niccoli et al., 2017, Beelen et al., 2017, Hermanky et al., 2017, Saudny-Unterberger et al., 1997, Weissberger et al., 2003, Martin-Salvador et al., 2016, Deer et al., 2019, Gade et al., 2019, Ortiz-Alonso et al., 2020, Pedersen et al., 2019) reported mean overweight (≥25) BMI; three studies reported mean obese (≥30) BMI in at least one arm (Torres-Sanchez et al., 2017, Files et al., 2020, López-López et al., 2019). One study reported data on ethnicity (Deer et al., 2019).

Two studies (McCullagh, 2017, Ortiz-Alonso et al., 2020) reported frailty prevalence in control and intervention arms by recognised definitions; a third reported mean frailty indices (Braun et al., 2019). Table 6.1-1 shows included studies' details; full study characteristics and results are available online (Appendix 8.7.2, Appendix 8.7.3). Table 6.1-2 shows effect sizes separated by interventions and outcomes.

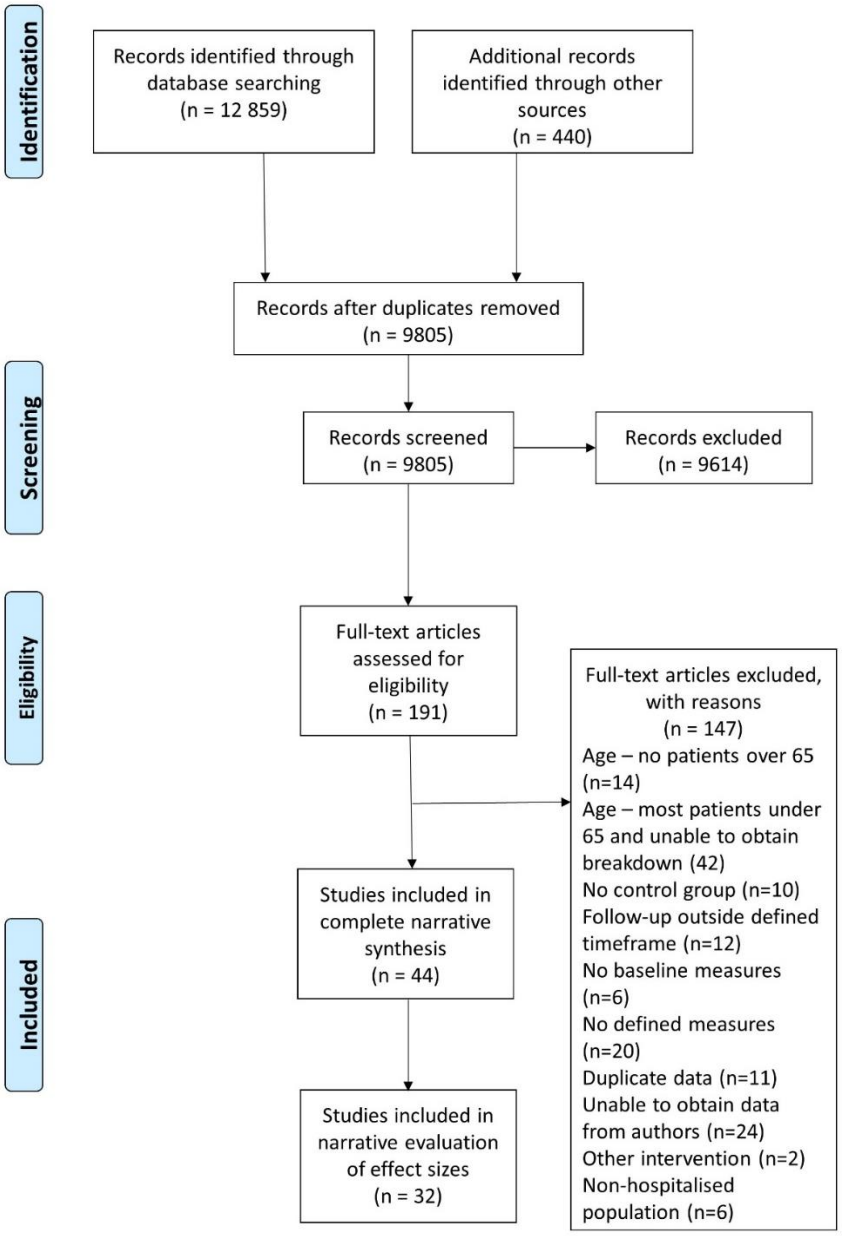

Figure 6.1-1 – Flowchart demonstrating identification of included studies.

Table 6.1-1 – Characteristics of all studies included in narrative synthesis.

All stages of screening and inclusion/ exclusion were performed in duplicate. Reasons for exclusion of articles reviewed as full texts are specified.

Author, date	Setting	N (control/ intervention)	Intervention	Outcomes
Physical activity				
Busch, 2012	Cardiac surgery	64/ 57	Resistance and balance training	TUG 6MWT Knee extension
Blanc-Bisson[b], 2008	Geriatric medicine	24/ 22	Early physiotherapy	Handgrip
Braun, 2019	Geriatric medicine	18/ 17	Augmented Prescribed Exercise Programme	Gait speed TUG 6MWT
de Morton, 2007	General medicine	126/ 110	Physiotherapy-designed exercises	TUG
Deer, 2019	General medicine	20/ 21	Chair-based and resistance exercise	SPPB DXA FFM
Fiore[b], 2017	Elective colorectal surgery	22/ 25	Early mobilisation	6MWT
Giangregorio, 2009	Orthopaedic rehabilitation	7/ 14	Body weight supported treadmill training	TUG
Henriksen[b],, 2002	Elective colorectal surgery	12/ 13	Enhanced recovery	Handgrip Knee extension
Houborg, 2006	Elective colorectal surgery	59/ 60	Strength training programme	Gait speed Handgrip strength Knee extension
Jones, 2006	General medicine	80/ 80	Individualised progressive exercise	TUG
Martínez-Velilla, 2019	Geriatric medicine	185/ 185	Multi-component physical exercise	Gait speed SPPB Handgrip
McCullagh, 2017	General medicine	95/ 95	Augmented prescribed exercise programme	Gait speed SPPB Handgrip

McGowan [b], 2018	Acute medicine for older people	25/ 25	Pedal exerciser	Knee extension Knee flexion
Moseley, 2009	Orthopaedic rehabilitation	80/ 80	Weight-bearing exercise	Gait speed Knee extension
Ortiz-Alonso, 2019	Geriatric medicine	131/ 150	Chair-based exercise and walking	SPPB
Opasich, 2010	Cardiac surgery	80/ 160	Individualised physical training programme	TUG 6MWT
Prasciene, 2019	Cardiac surgery	15/ 14	Balance and resistance training	SPPB 6MWT
Rahmann, 2009	Elective orthopaedic	20/ 24	Aquatic physiotherapy	TUG Knee extension
		24/ 21	Water exercise	TUG Knee extension
Raymond, 2017	Geriatric medicine	232/ 236	High intensity group exercises	TUG
Said [b], 2012	Geriatric rehabilitation	24/ 22	Enhanced physical activity	TUG
Said [b], 2018	Geriatric rehabilitation	93/ 98	Multimodal exercise programme	Gait speed TUG
Sano, 2018	Elective orthopaedic	41/ 40	Seated side tapping training	Gait speed TUG Knee extension Knee flexion
Schwenk, 2014	Geriatric rehabilitation	74/ 74	Individualised physical training programme	Gait speed Handgrip
Sherrington, 2003	Orthopaedic rehabilitation	39/ 41	Weight-bearing exercise	Gait speed Knee extension
Tal-Akabi[b],' 2007	Orthopaedic rehabilitation	29/ 33	High intensity exercise	TUG
Torres-Sánchez, 2017	Respiratory	29/ 29	Pedal exerciser	Knee extension
Wnuk, 2016	Vascular	16/ 15	Backward walking	6MWT
		16/ 16	Forward walking	6MWT
Nutrition				
Beelen, 2017	General medicine	39/ 36	Protein-enriched familiar foods	SPPB Handgrip Knee extension

Bouillanne, 2018	Geriatric rehabilitation	14/ 13	Citrulline amino acid	DXA ASMM
Deer, 2019	General medicine	20/ 20	Whey protein	SPPB DXA FFM
		20/ 20	Whey protein and exercise	SPPB DXA FFM
Ekinci, 2016	Orthopaedic surgery	37/ 38	Beta-hydroxy-beta-methylbutyrate	Handgrip
Files, 2020	Critical care	11/ 11	Nitrate-rich beetroot juice	SPPB
Gade, 2019	General medicine	82/ 83	Protein-enriched milk supplement	Gait speed Handgrip BIA FFM
Hermanky, 2017	Orthopaedic surgery	20/ 20	Nutritional consultation and exercise	Handgrip BIA FFM
Niccoli, 2017	Geriatric medicine	26/ 26	Whey protein	Gait speed TUG Handgrip Knee extension
Ogasawara, 2018	Respiratory medicine	21/ 21	EPA-enriched oral nutritional supplements	BIA SMI
Pedersen, 2019	General medicine	42/ 43	Protein and exercise	Gait speed Handgrip
Saudny-Unterberger, 1997	Respiratory medicine	16/ 17	Oral nutritional supplements	Handgrip
Pharmaceutical				
Deer, 2019	General medicine	20/ 19	Testosterone	SPPB DXA FFM
Hedström, 2004	Orthopaedic surgery	9/ 11	Growth hormone	Knee extension DXA LBM
Sloan, 1992	Orthopaedic surgery	14/ 15	Nandrolone	BIA FFM
Weissberger, 2003	Orthopaedic surgery	16/ 17	Growth hormone	Knee flexion CT thigh CSA
Zhang, 2019	Orthopaedic surgery	33/ 44	EPO injections (females)	DXA ASM
		25/ 39	EPO injection (males)	DXA ASM
Neuromuscular Electrical Stimulation				
López-López, 2019	General medicine	47/ 48	NMES and exercise combined	SPPB

Martin-Salvador, 2016	Respiratory medicine	20/ 24	Exercise and NMES combined	Knee extension
Zinglersen, 2018	Geriatric medicine	48/ 20	Chair-based functional exercise	Gait speed
		8/ 12	NMES and functional training	Gait speed

N=Participant numbers; TUG=Time up and go; SPPB=Short Physical Performance Battery; 6MWT=Six-minute walk test; DXA=Dual-Energy X-Ray Absorptiometry; ASMM=Appendicular Skeletal Muscle Mass; BIA=Bioelectrical Impedance Analysis; FFM=Fat Free Mass; SMI=Skeletal Muscle Index; LBM=Lean Body Mass; CT= Computed Tomography; CSA=Cross-sectional Area; LoS=Length of hospital Stay; COPD=Chronic Obstructive Pulmonary Disease

*= *Studies where insufficient information was available to estimate the SMD*

[b]*Unpublished data*

Table 6.1-2 – Summary of intervention effect by intervention type, outcome type, and effect size.

		Physical performance				Muscle strength			
		Effect size*	N (con/ exp)	Risk of Bias¥	Study	Effect size*	N (con/ exp)	Risk of Bias¥	Study
Physical activity	Strength and balance training	+ ++ ++ +++	93/98 64/57 20/ 21 15/ 14	+ +/- - -	Said, 2018 Busch, 2012 Deer, 2019 Prasciene, 2019	-	64/57	+/-	Busch, 2012
	Early/ increased mobilisation, or additional physiotherapy	- - ++ ++ -	24/ 22 22/ 25 16/ 16 126/ 110 131/ 150	- + +/- - -	Said, 2012 Fiore, 2017 Wnuk, 2016 de Morton, 2007 Ortiz-Alonso, 2020	- +++	24/ 22 12/ 13	- -	Blanc-Bisson, 2008 Henriksen, 2002
	Water exercise and physiotherapy	+	20/ 24	+/-	Rahmann, 2009	+	20/ 24	+/-	Rahmann, 2009
	Seated side tapping	+++	41/ 40	-	Sano, 2018	-	41/ 40	-	Sano, 2018
	Seated pedal exercises	No data				+ ++	25/ 25 29/ 29	- +/-	McGowan, 2018 Torres-Sanchez, 2017
	Progressive weight-bearing exercise	+ + +++	39/ 41 80/ 80 7/ 14	+/- +/- -	Sherrington, 2003 Moseley, 2009 Giangregorio, 2009	- -	39/ 41 80/ 80	+/- +/-	Sherrington, 2003 Moseley, 2009

	Individualised physical training programme	- + + +++ +++	95/ 95 74/ 74 80/ 160 185/ 185 18/ 17	+/- +/- - +/- +/-	McCullagh, 2017 Schwenk, 2014 Opasich, 2010 Martínez-Velilla, 2019 Braun, 2019	- - +++	95/ 95 74/ 74 185/ 185	+/- +/- +/-	McCullagh, 2017 Schwenk, 2014 Martínez-Velilla, 2019
Nutrition	Protein-enriched foods	+++ ++	26/ 26 20/ 20	+/- -	Niccoli, 2017 Deer, 2019	+ +++	39/ 36 26/ 26	+/- +/-	Beelen, 2017 Niccoli, 2017
	Protein and exercise	+++	20/ 20	-	Deer, 2019	+	42/ 43	-	Pedersen, 2019
	β-Hydroxy-β-MethylButyrate	No data				-	37/ 38	+/-	Ekinci, 2016
	Oral nutritional supplementation and snacks					+++	16/ 17	+/-	Saudny-Unterberger, 1997
	Nutrition consultation combined with exercise					+	20/ 20	+/-	Hermanky, 2017
Drugs	Testosterone	+++	20/ 19	-	Deer, 2019	No data			
	Growth hormone	No data				-	9/ 11	-	(Hedström et al., 2004)
NMES	NMES in combination with exercise	+++	47/ 48	+/-	López-López, 2019	+	20/ 24	+/-	Martin-Salvador, 2016

N=Participant numbers; con=comparison group; exp=intervention group; NMES=Neuromuscular electrical stimulation

**=Effect sizes categorised as: no effect [-] (≤0.1), small [+] (0.2 – 0.4), medium [++] (0.5 – 0.7), or large [+++] (0.8 or greater)*

¥=Risk of Bias categorised according to overall risk as: low [+], some concerns [+/-], or high [-]

6.1.4.3 Physical activity interventions

Most studies (61%; 27/44) reported physical activity interventions. Eighty-nine percent (24/27) included physical performance (Wnuk et al., 2016, Sherrington et al., 2003, Rahmann et al., 2009, de Morton et al., 2007, Schwenk et al., 2014, Giangregorio et al., 2009, Zinglersen et al., 2018, Moseley et al., 2009, Busch et al., 2012, Raymond et al., 2017, McCullagh, 2017, Martínez-Velilla et al., 2019, Jones et al., 2006, Fiore et al., 2017, Tal-Akabi et al., 2007, Houborg et al., 2005, Said et al., 2018, Sano, 2018, Said et al., 2012, Opasich et al., 2010, Braun et al., 2019, Deer et al., 2019, Ortiz-Alonso et al., 2020, Prasciene et al., 2019) and 44% (12/27) included muscle strength (Sherrington et al., 2003, Rahmann et al., 2009, Schwenk et al., 2014, Torres-Sanchez et al., 2017, Moseley et al., 2009, Busch et al., 2012, Martínez-Velilla et al., 2019, Blanc-Bisson et al., 2008, Henriksen et al., 2002, Houborg et al., 2005, Sano, 2018, McGowan et al., 2018). One study reported muscle quantity change (Deer et al., 2019), a multi-arm trial including nutritional and pharmaceutical interventions. Trials were conducted in various settings including elective orthopaedic (Rahmann et al., 2009, Sano, 2018), colorectal (Fiore et al., 2017, Houborg et al., 2005, Henriksen et al., 2002), orthopaedic rehabilitation (Sherrington et al., 2003, Giangregorio et al., 2009, Moseley et al., 2009, Tal-Akabi et al., 2007), vascular (Wnuk et al., 2016), and cardiac surgery (Busch et al., 2012, Opasich et al., 2010, Prasciene et al., 2019), and geriatric (Schwenk et al., 2014, Zinglersen et al., 2018, Raymond et al., 2017, Martínez-Velilla et al., 2019, Said et al., 2018, Said et al., 2012, Blanc-Bisson et al., 2008, McGowan et al., 2018, Braun et al., 2019), respiratory (Torres-Sanchez et al., 2017), and general medicine (de Morton et al., 2007, McCullagh, 2017, Jones et al., 2006, Deer et al., 2019).

A range of physical activity interventions were trialled; evidence for effect was limited. Interventions included strength and balance training (Busch et al., 2012, Tal-Akabi et al., 2007, Houborg et al., 2005, Said et al., 2018, Deer et al., 2019, Prasciene et al., 2019), early and/or increased mobilisation (Wnuk et al., 2016, Fiore et al., 2017, Said et al., 2012, de Morton et al., 2007, Blanc-Bisson et al., 2008, Henriksen et al., 2002), group exercise (Raymond et al., 2017), water exercise/physiotherapy (Rahmann et al., 2009), chair-based exercise (Zinglersen et al., 2018, Deer et al., 2019), seated side-tapping (Sano, 2018), pedal exercisers (Torres-Sanchez et al., 2017, McGowan et al., 2018), and progressive weight-bearing exercise in orthopaedic rehabilitation (Sherrington et al., 2003, Giangregorio et al., 2009, Moseley et al., 2009), using specialised harnesses where appropriate. An individualised multimodal physical training programme involving resistance exercise using machines and/or weights and gait/balance training substantially improved physical performance (gait speed and SPPB) and muscle strength in one of the largest studies (Martínez-Velilla et al., 2019). Other trials of individualised physical training programmes (strength with or without aerobic exercise stratified by frailty/functional status) showed small effects on physical performance (Schwenk et al., 2014, McCullagh, 2017, Jones et al., 2006, Opasich et al., 2010, Braun et al., 2019). Differences may relate to how interventions were delivered or adherence. The trial with the largest effect size reported adherence rates of 83.4 – 95.8% (≥90% exercises successfully performed each session) (Martínez-Velilla et al., 2019) compared to 59.7% (>3 sessions attended per week; offered daily) in another (Schwenk et al., 2014).

Interventions that ameliorated reductions in physical performance in trial populations included backward walking (Wnuk et al., 2016), progressive exercises stratified by frailty

(Opasich et al., 2010), resistance and balance training (Busch et al., 2012),, chair-based resistance exercise (Deer et al., 2019), individually progressed lower limb and core strengthening exercise (McCullagh, 2017), individualised progressive resistance, balance, and walking exercises (Martínez-Velilla et al., 2019), and seated side-tapping (Sano, 2018). Interventions that ameliorated reductions in muscle strength included pedal exercise (Torres-Sanchez et al., 2017), individualised progressive resistance, balance, and walking exercises (Martínez-Velilla et al., 2019), and early mobilisation with enhanced recovery after surgery (Henriksen et al., 2002). A high-intensity physiotherapy-led group exercise programme was as efficacious as individual sessions; group exercise resulted in improved therapist efficiency (Raymond et al., 2017). Group exercise was embedded into a multimodal physical training trial (Martínez-Velilla et al., 2019).

6.1.4.4 Nutritional interventions

Eleven nutrition trials were identified. Populations included orthopaedic surgery (Ekinci et al., 2016, Hermanky et al., 2017), geriatric (Niccoli et al., 2017, Bouillanne et al., 2018), general (Beelen et al., 2017, Deer et al., 2019, Gade et al., 2019, Pedersen et al., 2019), and respiratory medicine (Saudny-Unterberger et al., 1997, Ogasawara et al., 2018), and critical care (Files et al., 2020). Six studies reported physical performance change (Niccoli et al., 2017, Beelen et al., 2017, Gade et al., 2019, Pedersen et al., 2019, Deer et al., 2019, Files et al., 2020), seven muscle strength change (Beelen et al., 2017, Ekinci et al., 2016, Hermanky et al., 2017, Saudny-Unterberger et al., 1997, Niccoli et al., 2017, Gade et al., 2019, Pedersen et al., 2019), and four muscle quantity change (Hermanky et al., 2017, Ogasawara et al., 2018, Bouillanne

et al., 2018, Deer et al., 2019). Most studies were small; only one included more than 45 patients per arm. Interventions included protein enriched foods (Niccoli et al., 2017, Beelen et al., 2017) or supplements (Deer et al., 2019, Gade et al., 2019, Pedersen et al., 2019), β-Hydroxy-β-MethylButyrate (Ekinci et al., 2016), oral nutritional supplementation (Saudny-Unterberger et al., 1997), Eicosapentaenoic Acid (Ogasawara et al., 2018), citrulline (Bouillanne et al., 2018), nitrate-rich beetroot juice (Gade et al., 2019), and nutritional consultation (Hermanky et al., 2017). Three trials combined nutritional consultation to reach specified caloric/protein intake) with strength/resistance training (Hermanky et al., 2017, Pedersen et al., 2019, Deer et al., 2019). One study of progressive strength training followed by immediate protein supplementation showed statistically significant improved handgrip strength (Pedersen et al., 2019). Statistically significant improvements in physical performance were demonstrated comparing all interventions in a multi-arm study to placebo, including whey protein with/without exercise (Deer et al., 2019).

6.1.4.5 Pharmaceutical interventions

Five trials involved pharmaceuticals; four in orthopaedic surgery populations. Pharmaceuticals included Growth Hormone (GH) (Weissberger et al., 2003, Hedström et al., 2004), steroid (nandrolone) (Sloan et al., 1992), testosterone (Deer et al., 2019), and erythropoietin injections (Zhang et al., 2020). All studies measured muscle quantity (DXA, CT, or BIA) and both GH trials measured muscle strength. The only study that measured physical performance was the multi-arm study including physical activity and nutritional interventions (Deer et al., 2019). One GH trial showed statistically significant amelioration in muscle

quantity loss by DXA (Hedström et al., 2004) and the other showed statistically significant amelioration in knee flexion strength loss (Weissberger et al., 2003). Adverse events were similar between control and intervention arms (Weissberger et al., 2003); one study showed slightly higher peripheral oedema rates amongst GH recipients (Hedström et al., 2004). The nandrolone trial did not report statistically significant results (Sloan et al., 1992). Erythropoietin induced a small statistically significant amelioration in muscle quantity loss after orthopaedic surgery, not related to haemoglobin changes. Testosterone was safe in the multi-arm study, with statistically significant amelioration in physical performance demonstrated comparing all intervention groups to placebo (Deer et al., 2019).

6.1.4.6 Neuromuscular electrical stimulation

Three trials involved NMES (Zinglersen et al., 2018, Martin-Salvador et al., 2016, López-López et al., 2019); all combined NMES with exercise. One trial (geriatric medicine population) tested functional training alone against functional training with NMES (Zinglersen et al., 2018). No statistically significant different change in gait speed between groups was demonstrated. Another trial (respiratory medicine population) showed significant lesser decline in knee extension strength with NMES (Martin-Salvador et al., 2016). The third trial (general medicine population) resulted in significant improvements in physical performance with NMES (López-López et al., 2019).

6.1.4.7 Risk of bias and certainty across studies

Figure 6.1-2 shows overall risk of bias across studies. Full risk of bias details are shown in the online appendix (Appendix 8.7.4). There were at least some concerns for overall risk of bias across most studies. Adherence to trial intervention was associated with lowest risk and selection of reported outcome with highest risk. Most common reason for high risk of bias related to randomisation processes. Over half of studies exhibited at least some concerns for selection of reported result. Table 6.1-3 shows assessment of certainty for two interventions (individualised physical training programmes and protein supplementation) across studies.

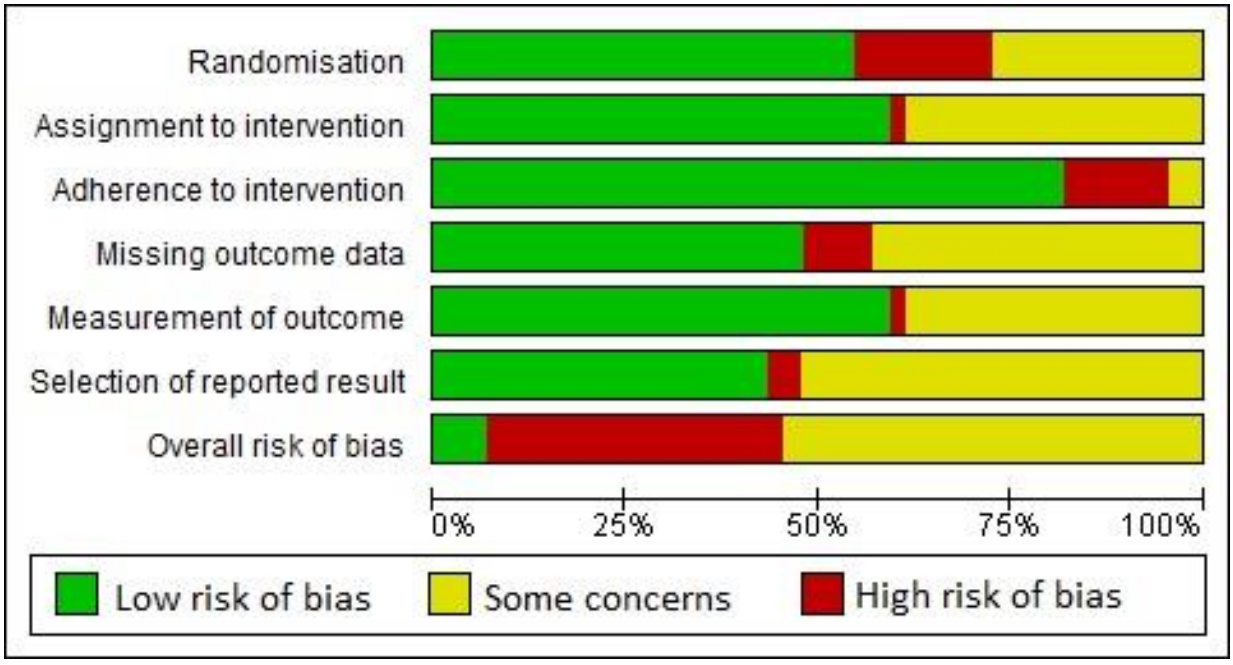

Figure 6.1-2 – Risk of bias results across all included studies.

Table 6.1-3 – GRADE domain certainty for individual physical training programmes and protein supplementation.

GRADE Domain	Certainty	Comments
Individual physical training programme		
Risk of bias	Moderate	RCTs assessed were mainly considered to have some concerns for overall risk of bias; no studies with high risk of bias.
Imprecision	Moderate	Meta-analysis of effect sizes across RCTs was not performed, although larger sample sizes in included studies.
Inconsistency	Low	Inconsistency of effect sizes across studies.
Indirectness	High	All but one study in geriatric medicine setting; all in older adults. All patients able to ambulate pre-admission and at risk of functional decline.
Publication bias	High	Publication bias of RCTs unlikely, particularly as mixed results presented. Inclusion of thesis and conference abstract for another physical activity intervention included.
Protein supplementation (with or without exercise)		
Risk of bias	Moderate	RCTs assessed were considered to have either low risk or some concerns for overall risk of bias.
Imprecision	Low	Overall small number of studies with low sample sizes.
Inconsistency	Moderate	Similar effect sizes demonstrated in small numbers of studies.
Indirectness	High	All studies performed in general/geriatric medicine setting in older adults.
Publication bias	High	Publication bias of RCTs unlikely, particularly considering identification of studies with low sample size.

6.1.5 Discussion

6.1.5.1 Interpretation of findings

Physical activity interventions were investigated more commonly than others. However, this mostly relates to studies with physical performance outcomes; only four trials not involving physical activity interventions measured physical performance (Niccoli et al., 2017, Beelen et al., 2017, Gade et al., 2019, Files et al., 2020). Conversely, many physical activity trials reported muscle strength change but only one measured muscle quantity change, a multi-arm study also involving nutritional/pharmaceutical interventions. Nutritional and pharmaceutical trials focused on muscle strength and quantity changes rather than physical performance. This suggests disconnect in how physical activity interventions are trialled compared to other interventions; physical performance declines may not be prioritised as organ insufficiency markers in need of urgent treatment.

Only nine trials reported muscle quantity change. This relates to historical reduced availability of feasible serial assessment tools; DXA, CT, and MRI remain gold-standard, but ultrasound is increasingly utilised (Cruz-Jentoft et al., 2018b, Wilson et al., 2019). As sarcopenia definition has developed, measures of muscle function are considered more important than muscle quantity (Cruz-Jentoft et al., 2018b). However, in acute sarcopenia, early muscle quantity declines may not be associated with muscle strength declines (Welch et al., 2018); preventing this may be important to prevent longer-term deteriorations. Additionally, muscle strength may be affected by fatigue/effort during acute illness making testing of efficacy/effectiveness

challenging (Van Ancum et al., 2017). Muscle quantity may be an appropriate treatment target in hospitalised patients; future trials of interventions for acute sarcopenia should consider incorporating in outcomes. Measurement of muscle quantity is also important to show biological effectiveness/mechanistic action.

We identified several physical activity interventions that stratified treatment protocols individually (e.g. by frailty) (McCullagh, 2017, Martínez-Velilla et al., 2019, Jones et al., 2006, Opasich et al., 2010, Braun et al., 2019). Most substantial and significant effects on muscle strength and physical performance were demonstrated in the highest reported adherence trial (Martínez-Velilla et al., 2019). Whilst this demonstrates high adherence of hospitalised older adults to complex trial designs is possible, effectiveness is expected to be reduced in clinical environments with limited compliance. Increasing mobilisation alone may be insufficient to prevent/treat acute sarcopenia (Wnuk et al., 2016, Fiore et al., 2017, Said et al., 2012, de Morton et al., 2007, Blanc-Bisson et al., 2008), although this is safe to do when possible and should be commended (Henriksen et al., 2002, Wnuk et al., 2016, Fiore et al., 2017, de Morton et al., 2007, Blanc-Bisson et al., 2008). Physical activity interventions can be multidimensional and include resistance exercise (Martínez-Velilla et al., 2019, Busch et al., 2012); it is safe and feasible to use machines/weights during acute phase of illness in hospitalised older patients (Busch et al., 2012, Martínez-Velilla et al., 2019, Schwenk et al., 2014). Pedal exercises (Torres-Sanchez et al., 2017, McGowan et al., 2018) and seated side-tapping (Sano, 2018) are simple, cheap, feasible, and potentially effective; these may be implemented as part of multidimensional stratified interventions. Group exercise may be as effective as individual exercise but more cost-effective (Raymond et al., 2017). Group exercise

has additional benefits of improving social interaction, and potentially improving motivation (Fuller et al., 2014) and adherence (Martínez-Velilla et al., 2019).

Several nutritional interventions were trialled. Although few trials showed statistically significant results, all trials were small and may have been under-powered for efficacy. Three trials combined nutritional intervention with physical activity (Hermanky et al., 2017, Deer et al., 2019, Pedersen et al., 2019). Research in chronic sarcopenia suggested additional protein supplementation may be most effective when combined with targeted physical activity i.e. resistance exercise (Martone et al., 2017). As inflammation and anabolic resistance are heightened with acute illness (Welch et al., 2018), greater doses (i.e. greater protein/amino acid intake) may be warranted in hospitalised older adults.

Few studies tested pharmaceuticals. There is suggestion from GH trials that this may be effective in ameliorating reductions in muscle quantity and strength (Hedström et al., 2004, Weissberger et al., 2003). Further research is needed, including longer-term outcomes. Benefits of GH supplementation need to be balanced against adverse effects, although supplementation was safe in dosages used in these small studies. Research is ongoing into novel pharmaceutical agents for use in acute and chronic sarcopenia (Hardee and Lynch, 2019). Studies assessing correlations between immune-endocrine biomarkers and phenotypic changes in muscle quantity, quality, or function will enable stratified treatments and direct potential drug pathways.

Trials of NMES showed conflicting results. NMES involves delivery of controlled electrical stimuli to superficial muscles via self-adhesive skin electrodes. These stimuli evoke muscle contractions, recruiting motor units and activating muscle fibres (Maffiuletti et al., 2019). NMES has been shown to ameliorate reductions in muscle quantity and function in healthy young volunteers during bedrest (Dirks et al., 2014). It is plausible that NMES may treat acute sarcopenia in hospitalised older adults. However, in establishing effectiveness in clinical practice, adherence, physical activity impact, and which muscle groups to stimulate should be considered.

6.1.5.2 What are the limitations of this review?

This review included hospitalised adults over 65 years-old. We excluded younger adults to focus towards most vulnerable patients, who are most likely to benefit from targeted interventions. More studies were excluded for participant age than were included (56 vs. 44). This suggests persistent bias against involvement of older people in clinical trials, particularly those with frailty. Considering we included search terms for older people in our search, it is likely more trials involving younger adults were not identified, as well as trials excluded through abstract screening. Trials conducted in younger adults may be useful when developing interventions for acute sarcopenia in older adults, but caution should be taken extrapolating results from younger less heterogeneous populations.

It is important to consider only three studies reported frailty status in both control and intervention arms (McCullagh, 2017, Braun et al., 2019, Ortiz-Alonso et al., 2020). Frailty was measured in intervention arms but rates were not reported in studies that stratified by frailty (Opasich et al., 2010, Schwenk et al., 2014). Whilst important measures, handgrip strength and gait speed alone may be insufficient to diagnose pre-morbid frailty during acute illness (Raymond et al., 2017). Recording levels of frailty prior to hospitalisation can ensure control and intervention arms are matched and enable sub-group analysis assessing treatment effect in individuals with and without frailty (McCullagh, 2017). Only one study reported ethnicity amongst participants (Deer et al., 2019). Normative values of muscle quantity may vary according to ethnicity (Silva et al., 2010), and muscle echotexture may differ (Melvin et al., 2014). Further research is needed to assess effects of genetics and environment on ethnic differences, and how these relate to differences in muscle function and responsiveness to interventions. Without information on ethnicity within published trials, it is not possible to assess for between group differences.

As described, majority of trials were small; many may have been underpowered to detect changes. Due to high heterogeneity in populations, interventions, and outcome measures, it was not possible to conduct meta-analyses. Some interventions that were not shown to be effective in small individual trials may be effective in larger powered studies. Additionally, most studies exhibited some concerns for risk of bias overall, and due to inconsistencies in effect sizes across different studies, there is limited certainty around true effect sizes. Many different outcome measures were also assessed across different RCTs. We consider that standardisation of assessment and outcome measures within geriatric medicine research will

enable greater ease of knowledge transfer, sharing of datasets, and future meta-analyses of RCTs in ageing.

It is important to consider that none of the included trials specifically included the presence of (acute or chronic) sarcopenia as inclusion criteria, or stratified treatment by sarcopenia. However, we consider that results of identified RCTs identified will be pivotal towards designing trials for prevention and/or treatment of acute sarcopenia. Acute sarcopenia is a rapidly progressing research area and therapeutic target. Twenty-two percent of studies (10/44) included in this review were published in the last 18 months. This demonstrates how rapidly progressive this area is, with increasing numbers of studies measuring muscle quantity and function as outcome measures.

6.1.6 Conclusion

Deteriorations in muscle quantity, strength, and physical performance are problematic in older adults following hospitalisation. However, insufficient evidence exists to enable targeted prevention/treatment strategies. A number of interventions have been trialled and shown to be safe for heterogeneous populations across various settings. Multidimensional physical activity interventions which are individually tailored (e.g. for frailty) have been trialled (Martínez-Velilla et al., 2019, McCullagh, 2017, Opasich et al., 2010); the trial with most substantial effect size reported excellent adherence (Martínez-Velilla et al., 2019). Large scale multi-arm studies assessing effectiveness of combined interventions including physical

activity (Martínez-Velilla et al., 2019, McCullagh, 2017, Sano, 2018, Torres-Sanchez et al., 2017, Opasich et al., 2010), NMES (Zinglersen et al., 2018), nutrition (Ekinci et al., 2016), and pharmaceuticals (Weissberger et al., 2003, Hedström et al., 2004) are warranted. Treatment may be most effective when stratified according to individual need. Treatment is likely to be guided by a combination of clinical and biological factors (e.g. immune-endocrine markers). Further research aimed at understanding pathophysiology of acute sarcopenia will enable risk stratification and targeted interventions.

6.1.7 References

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7 Discussion

7.1 Discussion

7.1.1 Defining acute sarcopenia

Acute sarcopenia is currently defined in line with chronic sarcopenia as muscle strength and quantity/quality below cut-offs more than two SDs below means within young healthy reference populations (Cruz-Jentoft et al., 2019, Welch et al., 2018). The defining characteristic of acute sarcopenia is that the incidence of this occurs within six months, normally following a stressor event (Cruz-Jentoft et al., 2019). However, this definition does not encompass the full spectrum of dynamic change that occurs during hospitalisation, but represents only the incidence of individuals meeting these "final stage" criteria for sarcopenia. Additionally, as demonstrated in Chapter 4.2, individuals may "develop" or "recover" from acute sarcopenia with the demonstration of only 1kg changes in handgrip strength above or below a prespecified cut-off. Such changes are unlikely to be clinically meaningful and likely fall within the realms of uncertainty of the test. Conversely, individuals with good baseline muscle quantity, quality, and function may experience large and clinically significant declines in these measures, but not meet criteria for acute sarcopenia according to a definition defined by cut-offs.

As has occurred with other conditions, the definition of acute sarcopenia is likely to continue to evolve over time. Acute sarcopenia is the last remaining acute organ insufficiency, and thus the last to achieve a clinically operational definition. However, much can be learnt from how

the definitions of other acute organ insufficiencies have developed. Acute Kidney Injury (AKI) as a term replaced the historically used “Acute Renal Failure”, in order to demonstrate the distinction between early and relative kidney damage from End-Stage Kidney Disease (Schrier, 2010). AKI is defined distinctly from Chronic Kidney Disease (CKD). Whilst CKD is defined by severity according to estimated Glomerular Filtration Rates below recognised cut-offs (Levey et al., 2005), AKI is defined by proportionate changes in serum creatinine in relation to baseline creatinine (Khwaja, 2012). A similar definition may be needed to define the spectrum of acute muscle insufficiency encountered in clinical practice (Welch et al., 2018). The challenge behind such a diagnosis will always be that it may not be possible to determine baseline measurements of muscle quantity, quality, and function. However, the same is often true with kidney disease, where monitoring of the trend remains important (Gaião and Cruz, 2010). Where there is uncertainty about acuity, disease should be assumed and treated as acute until proven otherwise.

It is recognised that there are other related and overlapping conditions with acute sarcopenia (Welch, 2021). Acute sarcopenia can be considered as part of a spectrum of acute muscle wasting disorders. Intensive Care Unit-Acquired Weakness (ICU-AW) is a recognised complication following admission to critical care (Kress and Hall, 2014). Similarly to acute sarcopenia, it is considered to arise due to a combination of immobility (Gruther et al., 2008) and acute surge in systemic inflammation (Witteveen et al., 2014). Following the COVID-19 pandemic, many patients who were previously fit with normal muscle quantity and function who survived admission to critical care with COVID-19 infection were found to develop profound skeletal muscle atrophy (de Andrade-Junior et al., 2021), and required intense

targeted multi-disciplinary rehabilitation (Welch et al., 2021). The longer-term effects of ICU-AW on these patients remain unknown, but are being evaluated in studies such as the Post-Hospitalisation COVID-19 (PHOSP-COVID) study (Brightling, 2020). The concept of induced frailty is described in Chapter 4.2. Induced frailty describes a state of increased vulnerability (Clegg et al., 2013) that arises due to the acute effects of illness itself (Hawkins et al., 2018). It is not muscle or organ-specific, but prevalence of the two conditions overlaps (Welch, 2021). I postulate that induced frailty may lead to increased risk of patients developing acute sarcopenia. Another related term that is commonly used to describe negative consequences of hospitalisation, particularly amongst older adults, is "deconditioning" (Falvey et al., 2015). There is no consensus definition on what encompasses deconditioning, and the term is commonly used informally outside of the research setting (British Geriatrics Society, 2020). Deconditioning is broadly considered to describe a multi-system process of decompensation due to the effects of illness, bedrest, or restrictive care-giving (Falvey et al., 2015, Guilcher et al., 2021). This may include acute sarcopenia and declines in muscle function (Falvey et al., 2015), but may also encompass other organ dysfunction e.g. skin (pressure ulceration) (British Geriatrics Society, 2020), urinary tract (urinary incontinence) (British Geriatrics Society, 2020), intestines (constipation) (British Geriatrics Society, 2020), cognitive spectrum disorders (Falvey et al., 2015), general fatigue (Guilcher et al., 2021), loss of motivation (Guilcher et al., 2021), and falls (British Geriatrics Society, 2020) secondary to instability and balance disorders.

Nevertheless, acute sarcopenia identified against the current EWGSOP2 criteria represents a significant outcome of hospitalisation. I have shown that one fifth of patients not meeting

criteria for sarcopenia at baseline subsequently meet criteria for acute sarcopenia. It is vital that this is considered in any studies researching sarcopenia within six months of hospitalisation or acute illness. As demonstrated in Chapter 4.2, hospitalisation is associated with bidirectional changes in sarcopenia status at follow-up. Thus, acute sarcopenia may be reversible in many cases. It is important to consider that only muscle quantity and not muscle quality cut-offs were used in the definition of acute sarcopenia described in Chapter 4.2. Although muscle quality is recognised within the EWGSOP2 definition, there are no currently agreed cut-off values for echogenicity (Cruz-Jentoft et al., 2019), and there is a recognition that echogenicity values may be operator and device-dependent, making standardised cut-off values challenging (Wilson et al., 2019). However, as described in Chapter 4.1, changes in echogenicity correlate with changes in muscle strength and gait speed across 13 weeks post-hospitalisation. Thus, effects on muscle quality rather than muscle quantity may be more significant in predicting recovery or declines in muscle function post-hospitalisation.

7.1.2 Techniques in muscle quantity, quality, and function assessment

As described in the introduction to this thesis, EWGSOP2 recommends CT, MRI, or DXA as gold-standard techniques in muscle quantity assessment (Cruz-Jentoft et al., 2019). However, none of these techniques can be performed at the bedside or outside of hospital settings, and all have limitations when performed serially including the time required to perform these procedures (associated with increased burden to patients and costs), and exposure to ionising radiation with CT and DXA. BIA is recommended as an alternative method, although

ultrasound is recognised as an emergent method (Cruz-Jentoft et al., 2019). There is currently no consensus upon what defines muscle quality. Mid-thigh or whole-body MRI, CT, or muscle biopsy are currently recommended within EWGSOP2 (Cruz-Jentoft et al., 2019). Both ultrasound and BIA offer alternative methods for estimation and monitoring of muscle quantity and quality, which can be used in a number of different clinical environments. In the studies presented in this thesis, assessments were made in the outpatient department, medical and surgical wards, critical care, clinical research facility, rehabilitation wards, and in participants' own homes. This demonstrates the utility of these techniques in being easily transportable and repeatable in an array of clinical settings.

There are currently multiple protocols for muscle quantity and quality assessment using ultrasound (Wilson et al., 2019, Perkisas et al., 2021). The ultrasound protocol used in this study (described in Chapter 2.1) was previously validated in community dwelling healthy young, healthy old, and frail older adults, but not in the presence of acute illness (Wilson et al., 2019). Chapter 2.2 describes the effect of position and exercise upon muscle quantity and quality measured using ultrasound quadriceps and whole-body BIA. Protocol standardisation was shown to be especially important with ultrasound in comparison to BIA. Bilateral Anterior Thigh Thickness (BATT) was shown to increase with participants in the sitting position. Differences between lying the participant supine and with the upper body tilted at 45°, but legs outstretched were not significant. This is particularly important in the acute setting, where it may not be possible to standardise position by lying the participant supine due to nausea or respiratory symptoms. The reclined position offers a pragmatic alternative that can be used in multiple clinical environments, but standardisation to exactly 45° is likely to be

challenging. These results suggest that small variations in the tilt of the upper body can be tolerated, so long as flexion of the hips and knees is avoided. BATT was also shown to increase following exercise. Considering this, the order that assessments are performed within studies should be standardised e.g. by measuring physical performance after muscle quantity and quality. The most recent SARCopenia through UltraSound (SARCUS) working group update specifically recommends standardisation of position across serial measurements of the rectus femoris, although there is no consensus on the position that should be used (Perkisas et al., 2021). The SARCUS working group previously recommended a 30 minute resting period prior to ultrasonography (Perkisas et al., 2018). This is now considered unnecessary as another study demonstrated minimal change after five minutes when changing from a standing to supine position (Lopez et al., 2019). SARCUS now recommends a five minute resting period between position changes prior to ultrasonography, although avoidance of exercise in the preceding 30 minutes is still advised (Perkisas et al., 2021).

Chapter 4.1 describes changes in muscle quantity, quality, and function during and following hospitalisation. Although changes were not significantly different at study population level, BATT appeared to be more sensitive to change than SMM measured by BIA. Similarly, gait speed was more sensitive to change than handgrip strength. Knee extension strength was not measured, but may be a more sensitive measurement of changes in muscle strength during hospitalisation, where the anti-gravity muscles are more likely to be affected (Hartley et al., 2020). However, as described in Chapter 4.1 and Chapter 4.2, changes were not uniform across the study population, with some participants experiencing improvements in individual

measurements, and others experiencing declines. Therefore, interpreting sensitivity to change at study population level may be inappropriate.

The measurement that did change most significantly within one week of hospitalisation was patient-reported physical function, as defined by the PROMIS T score. Lower step count was shown to be associated with lower patient-reported physical function, demonstrating that patients' own perceived physical function is predictive of objectively measured physical activity (Chapter 5.1). Similarly, although change in PROMIS T score at 13 weeks did not correlate with changes in muscle quantity, quality, or function measurements at 7 days, there was a moderate correlation between change in PROMIS T score at 13 weeks and change in SPPB at 13 weeks. This suggests that patient-reported physical function correlates with physical performance. However, as discussed in Chapter 4.1, the fact that PROMIS T score did not clearly relate to changes in muscle quantity, quality, or strength, suggests that there are likely to be broader causative pathways involved. Although PROMIS T score may not be specific to acute sarcopenia, the demonstration of sensitivity to change during hospitalisation suggests that it is likely to be an appropriate option to consider when embedding patient-reported outcomes within clinical study design.

7.1.3 Clinical and biological correlates of acute sarcopenia

Chapter 5.1 describes the association of baseline nutritional status and in-hospital step count with muscle quantity, quality, and physical function measurements. Worse nutritional status

at baseline was significantly associated with reduced muscle quantity and quality at baseline. This, therefore, suggests that nutritional status is likely to be significantly associated with the processes involved in the development of chronic sarcopenia. Importantly, there was a significant interaction between nutritional status, step count, and time, when considering muscle quantity measured by BIA (Janssen equation). Patients who had normal nutritional status at baseline but reduced step count in hospital were less likely to recover muscle quantity than those with higher step counts, and patients who were malnourished at baseline with higher step counts in hospital were more likely to recover muscle quantity than those with reduced step counts. This suggests that nutritional interventions are most likely to be effective when combined with physical activity interventions.

Chapter 5.2 describes the association of clinical features and systemic biomarkers with sarcopenia status. To ensure robust analysis and exclusion of features with high frequencies of missing data, this analysis focuses predominantly upon the prediction of sarcopenia status at 7 days post-admission/surgery. Clinical features that were shown to be associated with sarcopenia status at 7 days included the presence of COPD, and the prescription of steroids at any points during admission. This is consistent with previous research in healthy older adults, which has shown that hydrocortisone injection exacerbates loss of skeletal muscle mass with bedrest (Paddon-Jones et al., 2006). Dexamethasone has been shown to upregulate the muscle-specific E3 ubiquitin ligases MAFbx and MuRF-1 10-fold in rodent models (Bodine et al., 2001). This suggests that when considering how best to target interventions to treat or prevent acute sarcopenia, patients prescribed steroid medication may be a specific group most likely to benefit. This includes patients admitted with

exacerbations of COPD and asthma, as well as patients admitted with symptomatic COVID-19 (Welch et al., 2021). Delirium was also shown to be negatively associated with change in BATT to 7 days, suggesting that patients with delirium may be another group who would benefit from targeted interventions. Patients with cognitive impairment and delirium have often been excluded from trials of interventions to combat loss of muscle quantity and strength.

In Chapter 5.2, IL-1b was shown to be positively associated with sarcopenia status at 7 days. This suggests that IL-1b could potentially be used to risk stratify treatment and monitor response. Future studies should consider the role of anti-inflammatory treatment in ameliorating negative changes in muscle quantity and function, alongside increased protein intake.

7.1.4 The feasibility and acceptability of delivering acute sarcopenia research

Delivering acute sarcopenia research has unique challenges. These challenges need to be carefully considered when planning research trials and studies in complex heterogenous populations, but are not unsurmountable. Protocols need to be designed so that they are sufficiently pragmatic in clinical settings, but that ensure important aspects are standardised where possible. This should include standardisation of position and order of assessments, and consideration of expected completion rates when selecting assessments. Chapter 3.2 describes the completion rates of each assessment and recruitment and drop-out rates across the three cohorts. Physical performance had the lowest completion rates, whereas

ultrasound quadriceps had the highest completion rates. This should be considered when selecting measures within future studies. Although inclusion of all possible measures might be considered an option, this will be dependent on the design and purpose of the study. There is a risk that overburdening participants and researchers with measures could lead to lower completion rates overall. Completion rates were highest for ultrasound quadriceps, although it is important to note that the predominant reason for lower completion rates for BIA was due to exclusion of participants with cardiac devices from this procedure. BIA devices are increasingly approved for use on participants with cardiac devices, as performance has been shown to be safe. Completion rates for BIA and ultrasound quadriceps are, therefore, expected to be similar in future studies.

Completion rates for physical performance were significantly higher in elective surgery participants at baseline compared to medical participants. There were no significant differences in completion rates between cohorts across other visits. These results are as would be expected, as participants were in a state of clinical stability when recruited to the elective cohort. Completion rates were also higher at 13 week follow-up, which also represented a clinically stable state for most participants. These results are important in considering how completion rates might be affected when replicating studies conducted in community settings in acute hospital settings. It is important to consider that the majority of participants were recruited prior to the COVID-19 pandemic, and the protocol was subsequently amended following this so that only telephone follow-ups were conducted at 13 weeks. Whilst vaccination programmes have significantly improved outcomes from COVID-19, concerns around the risk of spreading COVID-19 to vulnerable older adults remain

(Antonelli et al.). Indeed, there is a recognition that reducing unnecessary contact between individuals can help to reduce the risk of infectious agents in general spreading (Chiu et al., 2020). The COVID-19 pandemic is likely to leave lasting impact upon how we conduct clinical studies and trials, particularly amongst people with underlying vulnerabilities (Richardson et al., 2020). Future research studies will need to continue to embrace these changes, with consideration of how recruitment and follow-up can be conducted remotely or in ways to minimise risk. Research trials are currently ongoing including remote measurement of handgrip strength and physical performance (Ni Lochlainn et al., 2021). Methodologically robust measurement of muscle quantity and quality is unlikely to be possible in studies with remote design. Pragmatic assessment using measurement of calf circumference by supplying participants or their carers with a tape measure may serve as an alternative. However, it is appreciated that the delivery of remote trials is likely to be more challenging when recruiting participants who are already frail and vulnerable. Participants surveyed prior to the COVID-19 pandemic reported very positively on being able to have follow-up assessments performed in their own homes Chapter 3.1. Whilst infection control procedures will need to be considered, it is imperative not to introduce bias by excluding participants who are unable to reliably access remote technology due to frailty, cognitive impairment, or cultural differences.

Importantly, the measures used within this study were shown to be acceptable to participants in Chapter 3.1. Ultrasound quadriceps, BIA, handgrip strength, and gait speed all scored highly on the multi-domain acceptability score. If anything, ultrasound and BIA were demonstrated to be slightly more acceptable than handgrip strength and gait speed, as they were associated with lower perceived burden to them as participants. Although the time that it takes to

perform ultrasound quadriceps assessments decreases with training and experience, it does take longer to perform than the other three procedures even with experienced sonographers. However, this was not clearly perceived by the participants, with no differences in opportunity costs reported between the different measures. Considering the higher completion rates of muscle quantity/quality assessment, the acceptability to participants, and the potential to demonstrate mechanisms of action of interventions, where physical function measurements may be affected by other factors, such as fatigue, it is logical to recommend that muscle quantity/quality assessment is incorporated into the design of clinical studies and trials for acute sarcopenia. At present, ultrasound and/or BIA provide the most practical methods of assessment.

Participation rates varied considerably between the three cohorts, and were highest amongst elective surgery participants, and lowest amongst emergency surgery participants. Drop-out rates were greatest amongst medical participants. This is especially important to consider when deciding upon required sample size to ensure that studies are adequately powered at follow-up, and upon estimating the numbers of potential participants required to approach. Ensuring that research is representative of the population is vitally important. Chapter 3.1 presents qualitative results on drivers for research participation and Chapter 3.2 presents qualitative results on reasons why patients declined to participate. Common drivers for research participation included the desire to help others and "give back", as well as educational value to themselves. Participants commonly declined to participate due to feeling too unwell or exhausted from the effects of their illnesses. However, some participants also described feeling "too old" to participate. Participation rates are likely to be highest when

participants are not suffering from the acute effects of illness, and research studies should aim to accommodate this as much as possible. Nevertheless, this is not always possible, and research studies that aim to intervene in the acute phase of illness will need to continue to recruit participants in the acute phase of illness.

7.1.5 Evidence for interventions

Chapter 6.1 summarises current evidence for interventions to ameliorate negative changes in muscle quantity, strength, and physical performance in hospitalised populations of older adults. Muscle quality was not specifically included within this review, but no trials were identified that obtained outcome measures for muscle quality, which is still a new and developing area. The broad range of interventions that were identified within this review demonstrates the multi-faceted nature to pathways that lead to declines in muscle quantity, quality, and function, and onto acute sarcopenia. However, effect sizes demonstrated were variable, and likely relates to targeting interventions broadly to heterogeneous populations. As described in Chapter 5.2 clinical features including COPD and prescription of steroids were particularly associated with the presence of sarcopenia at 7 days. These may serve as groups to focus interventions on in the future.

The conduct of these studies demonstrates that it is feasible to include muscle quantity, strength, and/or physical performance as outcome measures within clinical trials in hospitalised older adults. Few studies included measures of muscle quantity as outcome

measures, although this is expected to increase with wider utilisation of portable ultrasound and BIA devices. Broad intervention types that were identified include physical activity (Martínez-Velilla et al., 2019), nutrition (Niccoli et al., 2017, Deer et al., 2019), pharmaceutical (Deer et al., 2019), and neuromuscular electrical stimulation (NMES) (López-López et al., 2019). Interventions within these categories were also broad. The largest effect sizes were demonstrated for multicomponent physical activity programmes (Martínez-Velilla et al., 2019). When considering how best to deliver interventions to complex heterogeneous populations of older adults, multimodal programmes that take an individualised approach are likely to be most effective.

Exercise programmes should be graded and progressive according to individual function and goals progressing from bed-based, to chair-based (Sano et al., 2018), to assisted and dynamic standing (Braun et al., 2019) and machine-facilitated resistance training (Martínez-Velilla et al., 2019). Importantly, trials identified in this review demonstrated that implementation of these interventions in the acute phase of illness is feasible. Early implementation of interventions has potential to prevent declines in muscle quantity and function before they occur, rather than as a reactive approach, which may be beneficial towards promotion of long-term function. This also has potential to reduce length of stay in hospital, by reducing admission time for reactive rehabilitation programmes (Bachmann et al., 2010, Soh et al., 2021), leading to reduced costs overall (National Audit Office, 2016, Zhao et al., 2019), and reduced harm to patients from burdens of prolonged hospitalisation (e.g. healthcare associated infections) (Guest et al., 2020). There are, of course, many unknown factors. It is unclear what the impact of exercise interventions within the acute phase of illness could have

upon the illness itself. Physical activity has been repeatedly shown to be associated with improved immune function (Duggal et al., 2019), including enhanced natural killer cell activity (Nieman et al., 1993), preserved neutrophil migratory dynamics (Bartlett et al., 2016), and maintained thymic output (Duggal et al., 2018). However, some studies have also demonstrated temporary declines in immune function following strenuous exercise, although this is normally associated with improved function in the long-term (Peake et al., 2017). Exercise is known to induce release of endogenous opioids ("endorphins") from the anterior pituitary gland, notably β-endorphin and β-lipotropin (Saanijoki et al., 2018, Harber and Sutton, 1984), as well as high-circulating endocannabinoids, possibly secreted from the muscle itself (Hillard, 2018). Release of endorphins and endocannabinoids leads to reduced pain, perception and fatigue, and improved mood (Basso and Suzuki, 2017). Therefore, exercise interventions in the acute phase of illness could help to speed up recovery through improved immune function, reduced symptom burden, and improved motivation. Conversely, there may be negative effects from temporary immune function decline, exhaustion, and risks of injury (i.e. falls).

Nutritional interventions were predominantly trialled alone, rather than in combination with physical activity interventions. However, as evidenced by Chapter 5.1, nutritional status and physical activity interact. Currently, many researchers continue to work within silos of nutrition and/or physical activity interventions; collaboration between these fields is necessary. Whilst trials of single interventions may be simpler or cheaper in the short-term, these may be more costly in the long-term, as multiple distinct trials are necessitated, and the overall time to demonstrate outcomes can be prolonged. Increased systemic inflammation

and muscle disuse associated with acute illness and surgery is likely to promote a state of anabolic resistance, whereby higher protein or amino acid supplementation is necessary in order to promote muscle protein synthesis in response to exercise (Rittig et al., 2016, Breen et al., 2013). The PROT-AGE study group recommends that older adults with acute and chronic illness, without severe renal dysfunction, should consume 1.2 – 1.5g of protein/ kg body weight/ day (Bauer et al., 2013). For a 70kg person, this equates to 84 – 105g of protein, which equates to 3 – 4 chicken breasts (U.S. Department of Agriculture, 2019a), 7 – 9 eggs (U.S. Department of Agriculture, 2019b), or 5 – 6 bottles of commonly prescribed oral nutritional supplementation (Fortisip® Compact Protein: 18g/125mL bottle) (Nutricia, 2020). However, recent research has demonstrated that older adults do not perceive protein supplementation after exercise to be important, with half of respondents in a survey providing negative responses towards supplements (Hayes et al., 2021). It is perhaps unsurprising that older adults are commonly not meeting current recommendations during hospitalisation (van Bokhorst–de van der Schueren et al., 2012), thus, innovative methods of protein supplementation during hospitalisation are warranted. This could include providing protein-enhanced foods in a more appealing form such as fortified ice-cream or cheesecake (Wendin et al., 2021).

Pharmaceutical agents that were identified in Chapter 6.1 included GH (Weissberger et al., 2003, Hedström et al., 2004), testosterone (Deer et al., 2019), anabolic steroid (nandrolone) (Sloan et al., 1992), and erythropoietin (Zhang et al., 2020) injections. GH was shown to prevent loss of muscle quantity in one study (Hedström et al., 2004), and to prevent loss of muscle strength in another study (Weissberger et al., 2003). Testosterone was shown to

prevent declines in physical performance (Deer et al., 2019). Importantly, both GH and testosterone were shown to be safe in the dosages used in these trials (Deer et al., 2019, Hedström et al., 2004, Weissberger et al., 2003). GH is known to regulate muscle mass via IGF-1 (Bian et al., 2020). Indeed, for this very reason, GH has historically been used as a performance-enhancing drug in healthy adults (Saugy et al., 2006). GH administration has been shown to stimulate muscle protein synthesis (Fryburg and Barrett, 1993), and this effect is considered to be mediated mainly via stimulation of IGF-1 synthesis, which upregulates the PI3K/Akt pathway, which activates protein synthesis and inhibits protein degradation (Velloso, 2008). Testosterone has been shown to increase muscle mass, strength, and physical function in community-dwelling older adults (Srinivas-Shankar et al., 2010). Trials have been cautious due to concerns of risks of harm from long-term exogenous testosterone supplementation. However, no clear association between testosterone supplementation and prostate cancer, cardiovascular disease, or mortality has been demonstrated (Fernández-Balsells et al., 2010, Michaud et al., 2015). Regardless, these risks are likely to be less significant with short-term use to combat the negative effects of hospitalisation and illness. Importantly, the trial described in Chapter 6.1 included administration of testosterone to both men and women, and testosterone was shown to be safe in both sexes (Deer et al., 2019).

Whilst exercise and nutrition currently have the strongest evidence for efficacy in both acute and chronic sarcopenia, there is a recognition of the potential role for pharmaceutical agents (Zazzara et al., 2021). During acute illness, exercise may not always be possible, due to fatigue, impairments of consciousness, and safety concerns. Short-term use of pharmaceutical agents during hospitalisation to prevent or treat loss of muscle function may be more acceptable

than long-term administration. Further understanding of underlying mechanisms will enable the development of novel agents to directly target the pathways involved, or drug repurposing of licenced drugs that directly or indirectly target these pathways. Myostatin inhibitors are an example of a potential future pharmaceutical agents that could be trialled. Myostatin is a myokine secreted by skeletal muscle, which is a negative regulator of muscle mass. In rodent models of sepsis, myostatin deficiency has been shown to prevent muscle atrophy and inhibit increases in MAFbx and MuRF (Kobayashi et al., 2021).

Lastly, potential interventions could involve direct stimulation of skeletal muscles. Similar to pharmaceutical agents, these interventions are advantageous as being appropriate when exercise interventions are not feasible. However, these treatments will only target the specific muscle groups to which they are applied. Chapter 6.1 describes three trials of NMES with or without exercise in hospitalised older adults, with mixed results (López-López et al., 2019, Zinglersen et al., 2018, Martín-Salvador et al., 2016). Alternative methodologies which were not included in the review, but that have theoretical implications include massage, vibration, and low light laser therapy. Rodent models of hindlimb immobilisation have demonstrated an anabolic effect of massage in the form of cyclic compression loading (Lawrence et al., 2020). Vibration therapy (both whole-body and localised) has been trialled in community-dwelling older adults and shown to improve muscle strength and physical performance (Wu et al., 2020). Low level laser therapy has been shown to stimulate the mitochondrial respiratory chain, increasing adenosine triphosphate (ATP) production, and the synthesis of proteins. Low level laser therapy administered in conjunction with strength training has been shown to increase muscle strength in community-dwelling older adults (Toma et al., 2016).

7.1.6 Limitations

Limitations of individual studies and data interpretation are discussed within each chapter. Broadly, the most important limitation to the main study results is that the sample size was underpowered compared to the original planned sample size. I do not consider that this should significantly impact upon the interpretation of the feasibility (Chapter 3.2) and acceptability (Chapter 3.1) analyses. The under-recruitment compared to the original planned sample size arose due to unforeseen circumstances during the COVID-19 pandemic, rather than problems with the protocol itself or recruitment technique. However, it is possible that differences that were not statistically significant in (Chapter 4.1, Chapter 4.2, Chapter 5.1, or Chapter 5.2) may have been statistically significant in a larger powered study. Chapter 5.1 considers general trends of changes and not just statistical significance for this reason.

It is also unclear what impact the COVID-19 pandemic will now have upon the interpretation of results. The COVID-19 pandemic has had dramatic impact upon the lives of older adults, and the way that medicine is practiced across the world. During the COVID-19 pandemic, many countries implemented widespread policies ("lockdowns") to enforce social distancing and limit the spread of the virus. This led to many older people becoming socially isolated, and reducing their physical activity levels due to risks of contracting the virus (Salman et al., 2021). The long-term effects of this are still only just being realised. Many older adults have experienced significant declines in their physical function and general wellbeing, with increasing severity of frailty potentially making them more vulnerable to the effects of illness

(Shinohara et al., 2021). It is also recognised that COVID-19 in itself is a significant risk factor for the development of acute sarcopenia (Welch et al., 2021). Whilst many of the associations of COVID-19 with acute sarcopenia are not unique to this condition, hospitalisation with COVID-19 is associated with a specific combination of predisposing and precipitating factors that dramatically increase the risk of skeletal muscle insufficiency in all age groups. Severe COVID-19 is associated with substantial systemic inflammation (Huang et al., 2020), reducing muscle protein synthesis and increasing muscle protein breakdown. Prolonged bedrest is common in patients with severe disease, with isolation areas limiting opportunities for physical activity. Treatments given to patients with COVID-19 (e.g. dexamethasone (The RECOVERY Collaborative Group, 2020), and muscle relaxants in critical care (Luo et al., 2020)) may also precipitate acute sarcopenia (Paddon-Jones et al., 2006, Bodine et al., 2001). This is in addition to predisposing factors to severe illness with COVID-19 itself, which may impact on skeletal muscle outcomes, including age (Geriatric Medicine Research Collaborative and Covid Collaborative, 2021), frailty (Geriatric Medicine Research Collaborative and Covid Collaborative, 2021), Vitamin D deficiency (Hastie et al., 2020, D'Avolio et al., 2020), and obesity (Public Health England, 2020).

Of course, the COVID-19 pandemic has broadly impacted upon the way that medicine is practiced. Some of these changes may be positive to the recovery of older adults, such as changes to working patterns increasing availability of Allied Health Professionals across seven-day working (e.g. physiotherapists, dieticians, and speech therapists) (Thomas et al., 2020, Foster, 2020), and closer inter-specialty working. Other changes may be more detrimental towards skeletal muscle recovery. In the UK, patients are currently advised to

isolate prior to elective surgery, however, increased rates of respiratory complications have been demonstrated in patients who isolate compared to those do not. It is theorised that these negative effects may relate to reduced physical activity during the isolation period (CovidSurg Collaborative and GlobalSurg Collaborative, 2021). In unscheduled admissions, engagement with therapy and rehabilitation may be affected by reduced visiting of relatives/carers to support (Greenwood, 2020), and the use of personal protective equipment causing communication barriers (Hampton et al., 2020). These changes and the impact of them upon clinical care are likely to vary considerably between hospitals.

It is important to consider that the results of the studies presented in this thesis may not be broadly applicable to other populations. Sarcopenia is now recognised to affect adults of all ages (Cruz-Jentoft and Sayer, 2019). However, the main study presented included patients aged 70 years and older only. Therefore, it is not possible to extrapolate these results into younger age groups generally, where underlying predisposition may be different. Additionally, it is not known how results may differ in other populations (e.g. orthopaedic surgery). Elective colorectal surgery patients were chosen as a population whereby the effects of the operation upon limb muscle could be conceived as systemic rather than local, and as operations commonly performed for older adults of all genders. However, a significant number of these participants had colorectal cancer. Although cancer was normally localised, with operations performed with curative intent, it is unclear how this may have impacted upon the results of these studies.

Similar considerations about the broad applicability should be made for the results of the systematic review Chapter 6.1. This review included data for participants aged 65 years and older only; this can be considered both a strength and a limitation. The specific inclusion of older adults within this review ensured that interventions had been trialled in a representative population. On the other hand, interventions which may have been trialled in younger adults but of relevance to older adults may not have been identified. Effect sizes of interventions may have differed in trials including younger adults, and caution should be taken when extrapolating results into other clinical groups. Interventions that were trialled were heterogeneous, and, indeed, the populations included within individual studies were heterogeneous. For this reason, it was not possible to perform a meta-analysis of any specific intervention, and no definite conclusions about efficacy or effectiveness could be made.

7.1.7 Next steps

Further large-scale studies will enable enhanced understanding of the clinical utility of dynamic measurements of frailty and sarcopenia status in hospitalised older adults. The "Frailty and Outcomes Record in Clinical Environments: probable Sarcopenia, geriatric Evaluation, and Events (FORCE:SEE) Study" is a multi-centre study that aims to recruit 1000 hospitalised older adults from centres across the UK, of which I am the Chief Investigator. This study will involve recruitment of acutely hospitalised older adults, with assessment of handgrip strength and physical performance within 48 hours of admission and discharge. This study is designed to be pragmatic and feasible at scale. Dynamic measurements of mid-arm

and calf circumference will be made, but no skeletal muscle imaging or invasive studies will be completed. This study aims to assess the relationship between these measures (both at baseline and dynamic change) and routinely collected clinical information including 30-day readmissions, as well as patient-reported quality of life.

Longer-term follow-up studies will also enhance understanding of clinical trajectories of patients who experience dynamic changes in skeletal muscle quantity, quality, and function, and/or frailty status. Ethical approval has been obtained to enable record-linkage of participant data from the main study presented within this thesis with routinely collected data up until one year. This will enable assessment of the association of these changes with outcomes including hospital readmission and mortality.

The Post-Hospitalisation COVID-19 (PHOSP-COVID) study is a UK multi-centre study that involves follow-up of patients who had previously been hospitalised with COVID-19 at two timepoints (2 to 7.5 months post-discharge, and 10 to 14 months post-discharge) (PHOSP-COVID Researchers, 2020); I am presently involved in recruitment and follow-up to this study. Assessment at each of these two timepoints includes detailed questionnaire-based assessments of physical and cognitive function, handgrip strength, BIA, physical performance (SPPB and incremental shuttle walk), blood tests, and at some centres quadriceps muscle strength, ultrasound quadriceps, DXA, MRI, and/or muscle biopsies. This study is not unique to older adults, but includes hospitalised patients aged 18 years and older. The study does not incorporate assessment during the acute illness, but rather focuses on factors that predict

recovery. The results of this study are likely to be of broad relevance to hospitalised patients with severe illness generally, by assessing outcomes from a single illness across a heterogeneous population. Thus, effects and trajectories can be determined at an individual level.

Additionally, I am collaborating on another study which will include a sarcopenia sub-study. The "Duration of External Neck Stabilisation (DENS) Trial" will randomise patients with odontoid peg fracture to either early removal of a hard collar or standard care (Brennan, 2021). Serial measurements of handgrip strength and muscle quantity estimated by BIA will be performed as part of this trial. Assessments will be measured at admission, and at 12-week follow-up. This study will add further characterisation of dynamic changes in muscle quantity and function in a complex population.

I am also currently collaborating internationally on a large systematic review (SARCUS4) to determine the relationship between skeletal muscle imaging and clinical outcomes, with a specific focus on ultrasound imaging. This review will determine the relationship between skeletal muscle thickness, physiological and/or anatomical cross-sectional area, fascicle length, pennation angle, and echogenicity with broad clinical outcomes including but not limited to mortality, functional outcomes, nutritional status, and cognition.

Increasing understanding of mechanisms underlying acute sarcopenia remains a significant interest. However, I also consider that interventional studies should not be delayed whilst this

research is ongoing. Overall aims should be to enhance benefit to patient treatment and care, and early interventional studies are necessary in order to translate findings into clinical practice. Interventional study designs that also incorporate mechanistic studies through collection of biological specimens from participants will enable early identification of effective therapies, alongside enhanced understanding of biological mechanisms underpinning skeletal muscle changes and efficacy of treatment. This can lead on to identification of further novel treatment strategies, and enhanced stratification of treatment and monitoring of response. Skeletal muscle biopsies collected as part of the PHOSP-COVID study will enhance understanding of mechanistic determination of skeletal muscle recovery following severe illness. In addition, there may be as yet unmeasured biomarkers within serum and plasma samples saved as part of this research that may prove to be pertinent. There is potential to develop further collaborations into the identification of such biomarkers.

Lastly, the most important next step will be in the design of pragmatic clinical trials to enable the delivery of interventions to ameliorate negative changes in muscle quantity, quality, and function in hospitalised populations of older adults. Each chapter of this thesis provides invaluable information towards the design and conduct of such clinical trials: 1) Assessment of muscle quantity by ultrasound and/or BIA are especially acceptable, and feasible to conduct across multiple clinical settings; 2) Assessment of muscle quantity is important in terms of showing biological effectiveness, which may not be captured with subjective measurements; 3) Patient-reported outcomes should be integrated within trial design. Other outcome measures will be dependent upon the setting of the study. Initial steps in the delivery of complex clinical trials will involve single site feasibility and pilot studies, which

could be delivered as a single multi-arm study. Considering the results of Chapter 5.1, Chapter 5.2, and Chapter 6.1, interventions that should be considered either alone or in combination include multidimensional progressive exercise, protein supplementation, and pharmacological agents e.g. GH, myostatin inhibitors, DHEA.

7.1.8 Concluding remarks

Acute sarcopenia is the last remaining acute organ insufficiency. It currently remains under-considered in clinical practice. Early consideration and monitoring of muscle quantity, quality, and function in hospitalised patients would enable early detection and recognition. The studies presented in this thesis have shown that assessment of muscle quantity, quality, and function in heterogeneous older patients in multiple clinical environments are feasible and acceptable to patients themselves. In some, but not all, older patients muscle parameters decline during and following hospitalisation. In some cases, this leads on to patients newly meeting criteria for sarcopenia, even within short time periods. Patients on treatment with steroids are more likely to meet criteria for sarcopenia one week after hospitalisation or surgery. Malnutrition and reduced step count in hospital, as well as baseline GH levels may lead to increased risk. Further interventional studies are urgently warranted, in order to prevent devastating longer-term outcomes for patients.

7.1.9 References

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8 Appendix

8.1 Chapter 2.1 – Supplementary information

8.1.1 Criteria used to form Frailty Index

Deficit	Definition
Activity limitation	Positive Fried physical activity score
Anaemia and haematinic deficiency	As per local reference ranges (female Hb<115, male Hb<135) or on medication for haematinic deficiency
Arthritis	Patient reported (includes osteoarthritis and inflammatory arthritis)
Atrial fibrillation	Any history – paroxysmal, temporary, or permanent
Cerebrovascular disease	Vascular dementia or stroke disease
Chronic kidney disease	eGFR <60
Diabetes mellitus	Known history/ confirmed diagnosis
Dizziness	Patient reported
Dyspnoea	Patient reported
Falls	Two or more over previous year
Foot problems	Patient reported
Fragility fracture	Previous history
Hearing impairment	Need for hearing aids
Heart failure	Known history/ confirmed diagnosis
Heart valve disease	Known history
Housebound	Lawton instrumental ADLs
Hypertension	On treatment or recorded
Presyncope/ syncope	Patient reported (altered from “hypotension” in original eFI)
Ischaemic heart disease	Known history

Memory and cognitive problems	Any cognitive spectrum disorder including mild cognitive impairment, delirium, and dementia
Osteoporosis	On treatment or known history
Parkinsonism and tremor	Includes tremor of any cause – known history or on treatment
Peptic ulcer	Known history
Peripheral vascular disease	Known history
Polypharmacy	≥5 prescribed medications
Requirement for care	Formal carers
Respiratory disease	Any history of chronic disease e.g. asthma, COPD
Skin ulcer	Present history as per Mini Nutritional Assessment (MNA) – patient reported
Sleep disturbance	Patient reported
Social vulnerability	Lives alone
Thyroid disease	Known history
Urinary or faecal incontinence	Katz ADLs
Urinary system disease	Known history
Visual impairment	Wears glasses/ visual aids or on treatment for eye condition(s)
Weight loss and anorexia	Fried weight loss OR MNA weight loss OR MNA intake decline

8.1.2 Delirium assessment

a A disturbance in;			
i) Attention- reduced ability to direct, focus, sustain, and shift attention FROM; *20-1, MOYB, observation*	**Yes**	No	?
ii) Awareness (reduced orientation to the environment)	**Yes**	No	?

	FROM; *mRASS not 0, observation*			
b	The disturbance;			
	i) Develops over a short period of time (usually hours to a few days)	**Yes**	No	?
	Ii) Represents a change from baseline attention & awareness and iii) tends to fluctuate in severity during the course of the day	**Yes**	No	?
	FROM: *History*			
c	An additional disturbance in cognition (e.g. memory deficit, disorientation, language, visuospatial ability, or perception). FROM; *Describe a pen, describe the morning, AMTS questions, observation*	**Yes**	No	?
d	Exclusions- The disturbance in criteria A and C are;			
	i) Better explained by another pre-existing, established, or evolving neurocognitive disorder, or ii) Occur in the context of a severely reduced level of arousal such as coma. FROM; *mRASS not -4, -5*	Yes	**No**	?
e	There is evidence from the history, physical examination or laboratory findings that the disturbance is a direct physiological consequence of another medical condition, substance intoxication or withdrawal, or exposure to a toxin, or is due to multiple aetiologies. FROM*; Notes (likely to be yes as in hospital)*	**Yes**	No	
	Probable Delirium Diagnosis – all items a,b,c and e 'yes', plus d 'no'	**Yes**	**No**	
	Possible delirium diagnosis – if any '?' or e 'no'	Yes	No	

8.1.3 Acceptability questionnaire

Considering the testing of **handgrip strength**, please rate your agreement with the following:

	Strongly disagree (1)	Disagree (2)	Neither (3)	Agree (4)	Strongly agree (5)
I enjoyed participating in this test	◯	◯	◯	◯	◯
Minimal effort was required to participate in this test	◯	◯	◯	◯	◯
This test was unobtrusive	◯	◯	◯	◯	◯
I understand how this test works and its importance	◯	◯	◯	◯	◯
This test was not time-consuming	◯	◯	◯	◯	◯
This test is likely to have a positive impact on patients	◯	◯	◯	◯	◯
I felt confident that I could complete this test	◯	◯	◯	◯	◯

Considering the testing of **walking speed**, please rate your agreement with the following statements:

	Strongly disagree (1)	Disagree (2)	Neither (3)	Agree (4)	Strongly agree (5)
I enjoyed participating in this test	◯	◯	◯	◯	◯
Minimal effort was required to participate in this test	◯	◯	◯	◯	◯
This test was unobtrusive	◯	◯	◯	◯	◯
I understand how this test works and its importance	◯	◯	◯	◯	◯
This test was not time-consuming	◯	◯	◯	◯	◯
This test is likely to have a positive impact on patients	◯	◯	◯	◯	◯
I felt confident that I could complete this test	◯	◯	◯	◯	◯

Considering the testing of **muscle mass using ultrasound**, please rate your agreement:

	Strongly disagree (1)	Disagree (2)	Neither (3)	Agree (4)	Strongly agree (5)
I enjoyed participating in this test	◯	◯	◯	◯	◯
Minimal effort was required to participate in this test	◯	◯	◯	◯	◯
This test was unobtrusive	◯	◯	◯	◯	◯
I understand how this test works and its importance	◯	◯	◯	◯	◯
This test was not time-consuming	◯	◯	◯	◯	◯
This test is likely to have a positive impact on patients	◯	◯	◯	◯	◯
I felt confident that I could complete this test	◯	◯	◯	◯	◯

Considering **bioelectrical impedance analysis**, please rate your agreement with the following:

	Strongly disagree (1)	Disagree (2)	Neither (3)	Agree (4)	Strongly agree (5)
I enjoyed participating in this test	◯	◯	◯	◯	◯
Minimal effort was required to participate in this test	◯	◯	◯	◯	◯
This test was unobtrusive	◯	◯	◯	◯	◯
I understand how this test works and its importance	◯	◯	◯	◯	◯
This test was not time-consuming	◯	◯	◯	◯	◯
This test is likely to have a positive impact on patients	◯	◯	◯	◯	◯
I felt confident that I could complete this test	◯	◯	◯	◯	◯

Any further comments related to this study?

8.2 Chapter 2.2 – Supplementary information

8.2.1 Exercise protocol

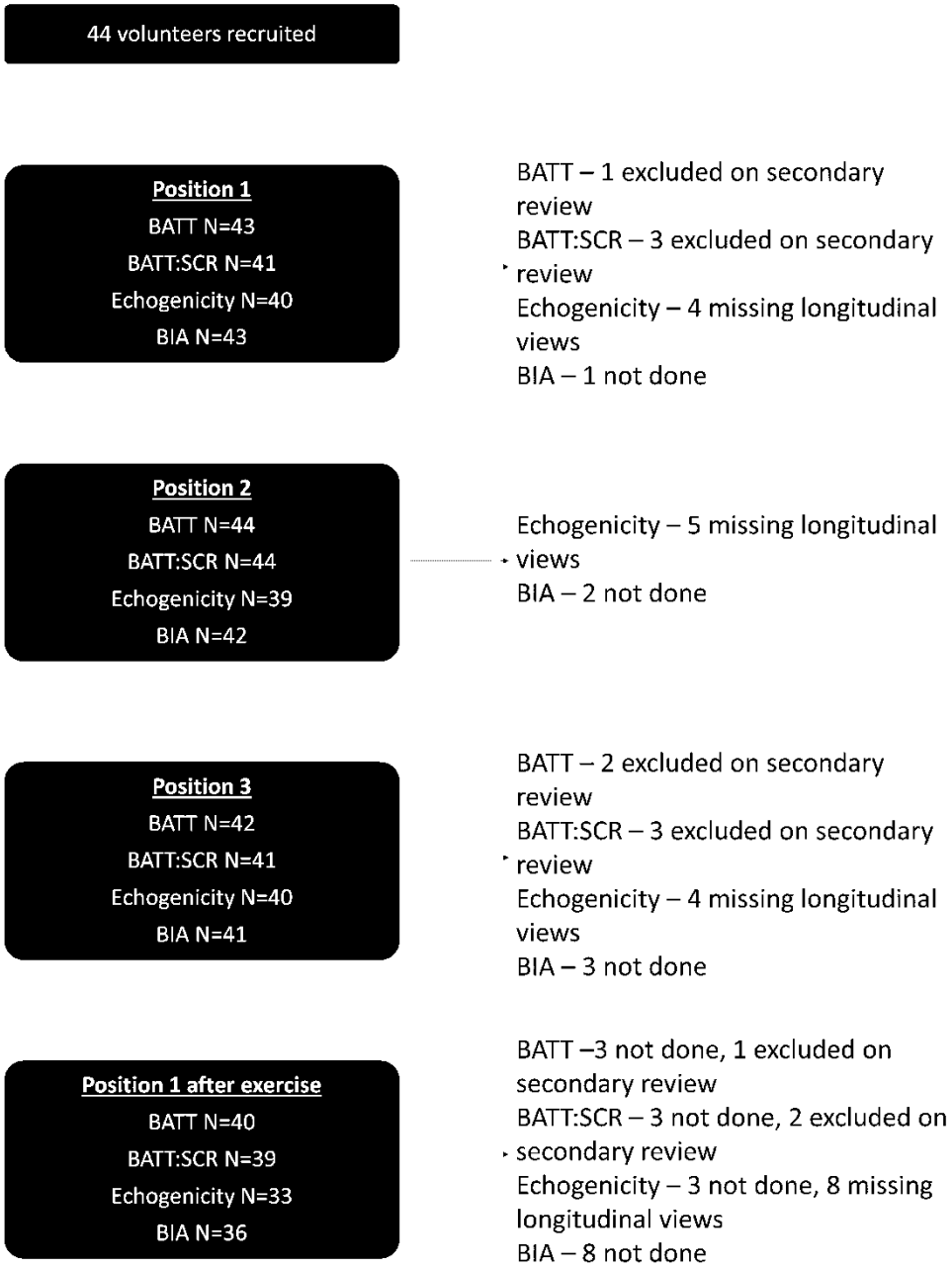
Participants were advised to complete the following exercises:

- 20 Star-jumps: Participants were advised to jump from a standing position with their arms by their side, to a wide legged stance with their legs separated and their arms in a "V" position above their heads. This was achieved through shoulder abduction and moving their arms sideways into the posture. Participants then jumped back into their original resting posture with their arms by their sides and their feet together in parallel. They were asked to repeat this 20 times.

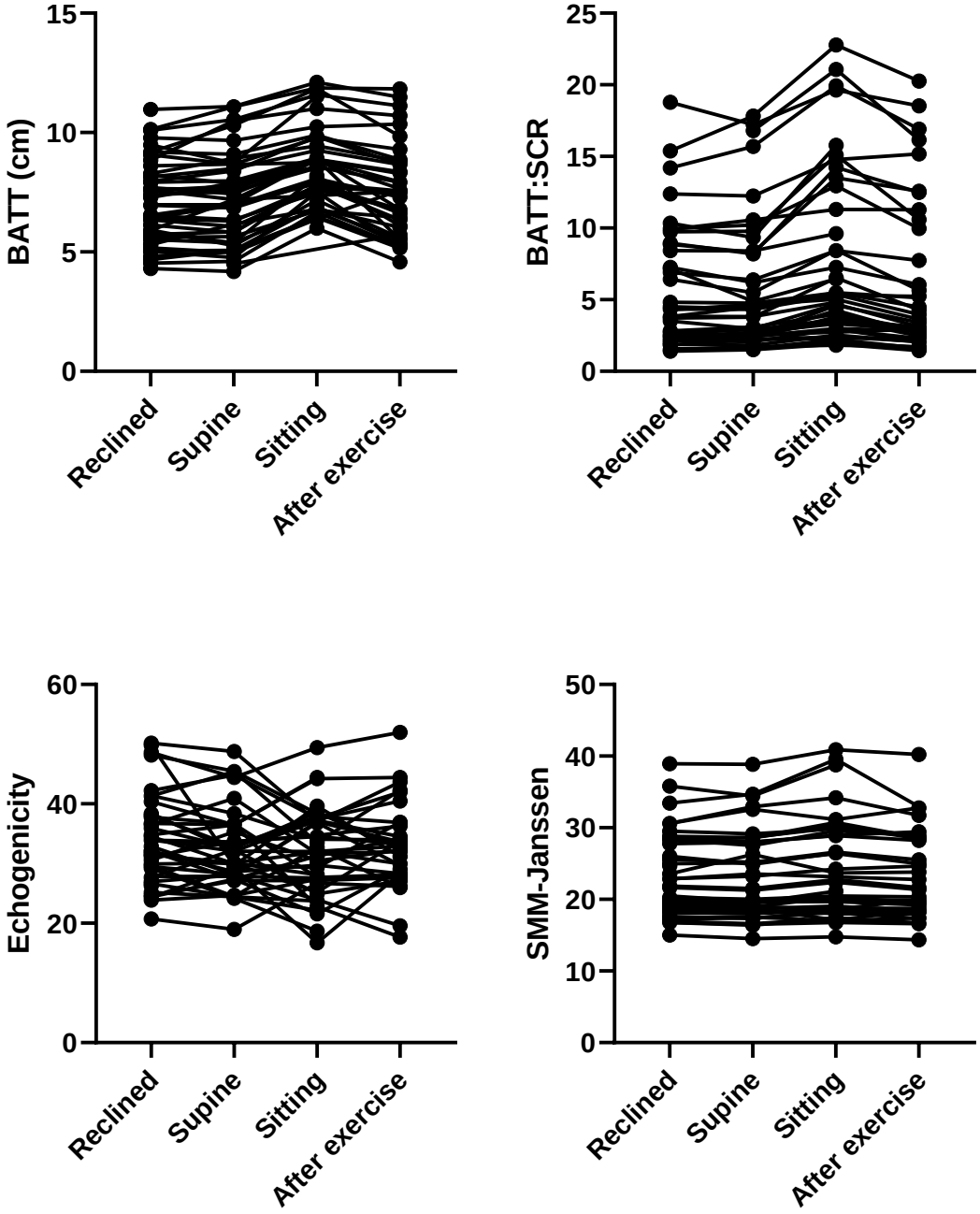
- 20 Squats: Participants were advised to stand with their feet shoulder width apart and squat down by bending their knees while moving their hips back, until their hips were parallel to or just below their knees. They were then advised the push themselves back to the standing position, keeping their knees and chest out, whilst pulling their hips up. They were asked to repeat this 20 times.

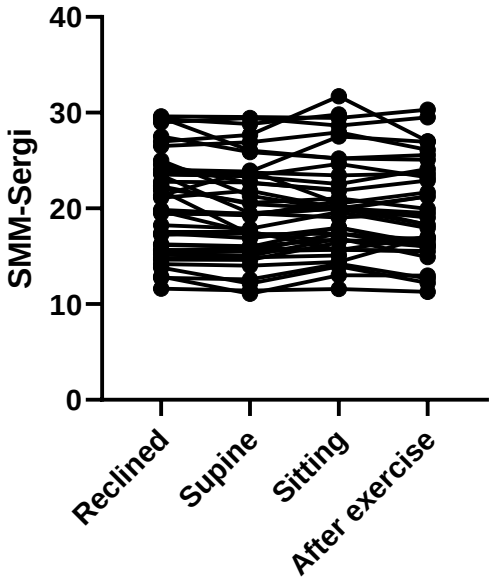
- 20 Burpees: Participants were advised to start in neutral standing position with their hands by their sides. They were then advised to squat down with their knees fully bent and their hands touching the floor, and from this position immediately push themselves forwards into a "plank" or "push-up" position. In this position, the toes remained touching the floor, and their body was flat but not touching the floor, with their hands on the floor underneath their shoulders, and their arms bent at the elbows. They were then advised to move back into the full squat position in a single movement, and from this position to jump up back into the standing position with their hands above their head. There asked to repeat this 20 times, or until they were unable to complete further burpees due to muscle soreness or fatigue.

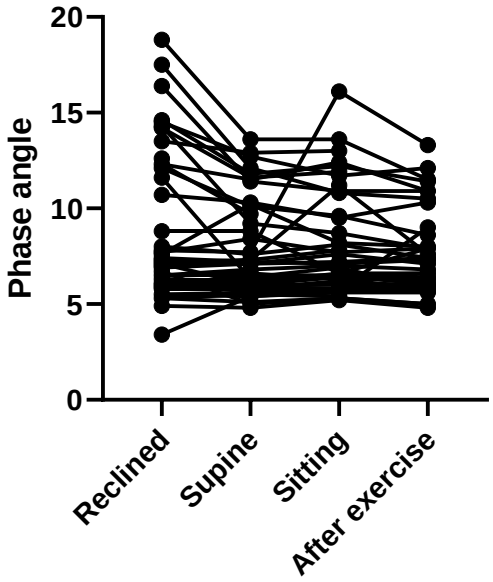
8.2.2 Missing data for each position/ repeated measure

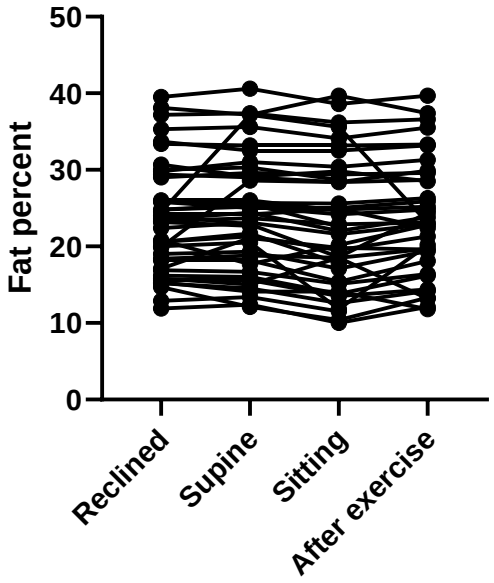


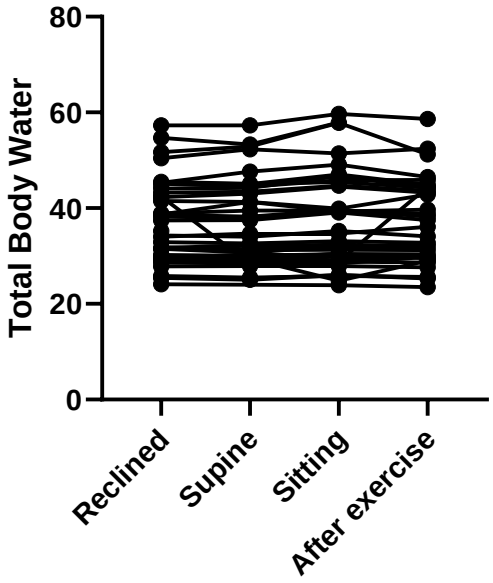
8.2.3 Individual participant changes with position and after exercise

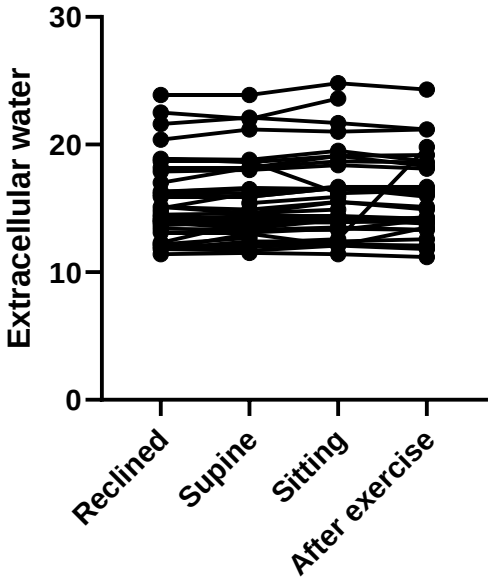


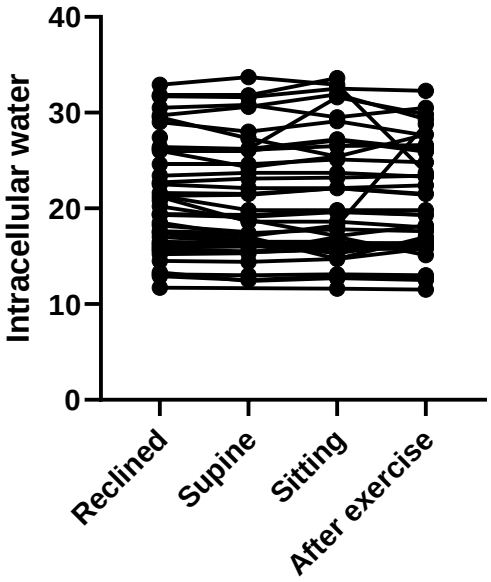

SMM-Sergi
40
30
20
10
0
Reclined
Supine
Sitting
After exercise


Phase angle
20
15
10
5
0
Reclined
Supine
Sitting
After exercise


Fat percent
50
40
30
20
10
0
Reclined
Supine
Sitting
After exercise


Total Body Water
80
60
40
20
0
Reclined
Supine
Sitting
After exercise


Extracellular water
30
20
10
0
Reclined
Supine
Sitting
After exercise


Intracellular water
40
30
20
10
0
Reclined
Supine
Sitting
After exercise

8.3 Chapter 4.1 – Supplementary information

Table 8.3-18.3-2: Research procedures performed at each timepoint and included within analysis separated by cohort.

Coloured squares designate timepoints where these were performed. Timepoints where these were not performed are shown in black.

	Elective surgery			Emergency surgery			Medical		
	Pre-operative	7 (+/-2) days post surgery	13 (+/-1) weeks post surgery	Within 48hr of surgery	7 (+/-2) days post surgery	13 (+/-1) weeks post surgery	Within 48hr of admission	7 (+/-2) days post admission	13 (+/-1) weeks post admission
Ultrasound quadriceps									
Bioelectrical impedance analysis									
Handgrip strength									
SPPB									
Gait speed									
PROMIS Physical Function									

Table 8.3-3: Equations used in calculation of Skeletal Muscle Mass (SMM) using bioelectrical impedance analysis.

In both equations: Height in cm; Sex 1=male, 0=female; Weight in kg; Resistance in Ω; Reactance in Ω

Skeletal Muscle Parameter	Equation
SMM-Sergi	= -3.964 + [0.227 × (height^2/resistance)] + (0.095 × weight) + (1.384 × Sex) + (0.064 × reactance)
SMM-Janssen	= [(height^2/resistance) × 0.401] + (Sex × 0.3825) + (Age × -0.071) + 5.102
Phase angle	= arctan(reactance/resistance) *"arctan" is the inverse trigonomic function (arc tangent) of the tangent function*

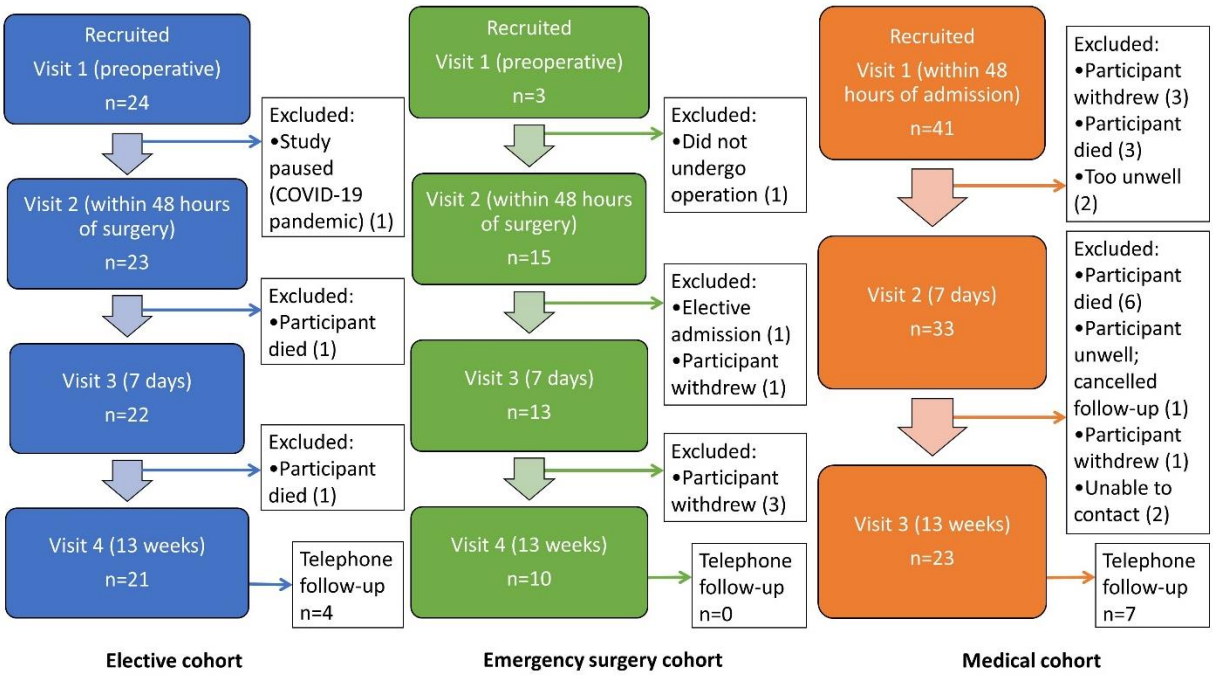


Figure 8.3-1 – Recruitment and drop outs of participants across visits for each patient cohort

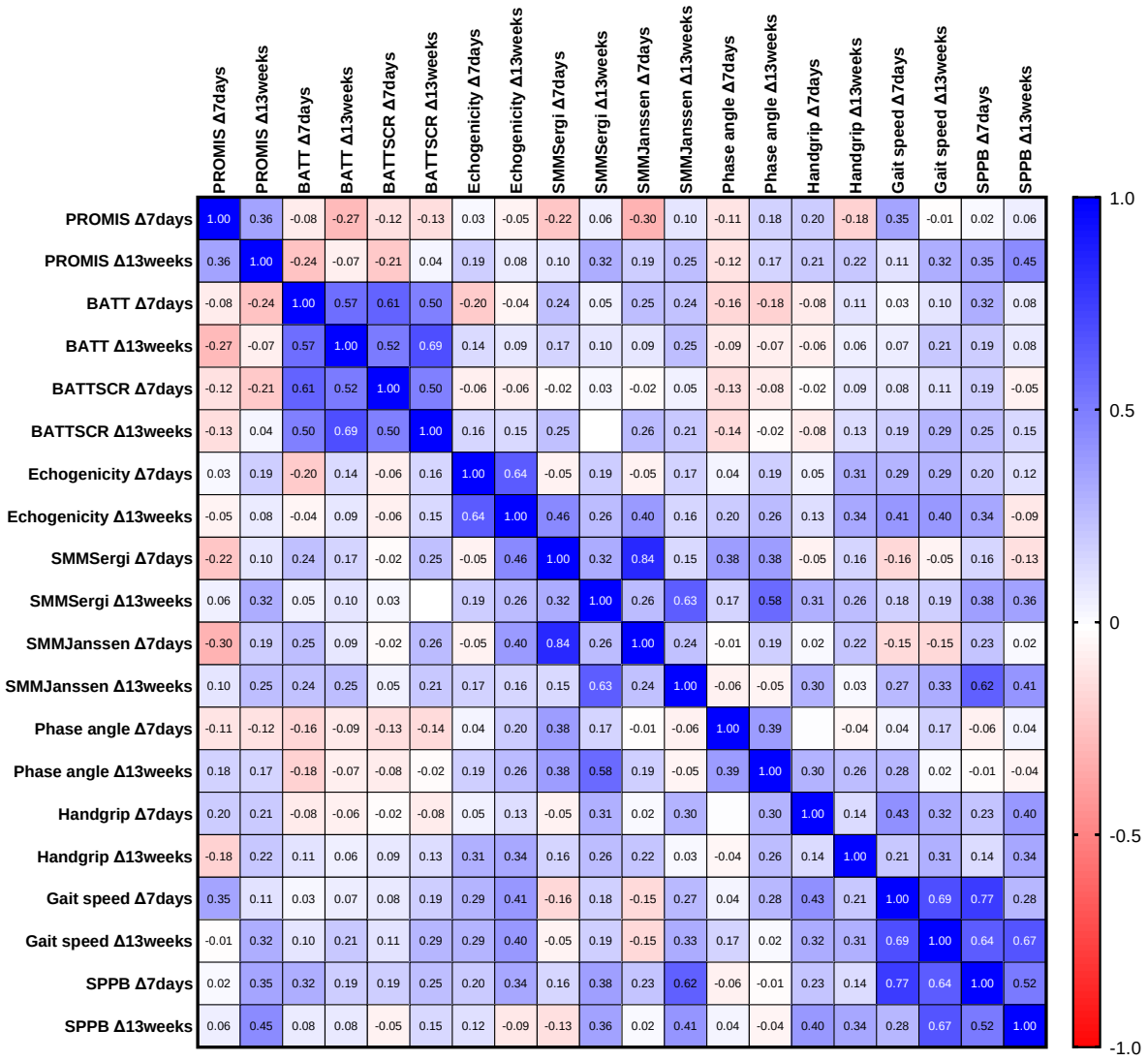


Figure 8.3-2 – Correlation matrix using Spearman correlations

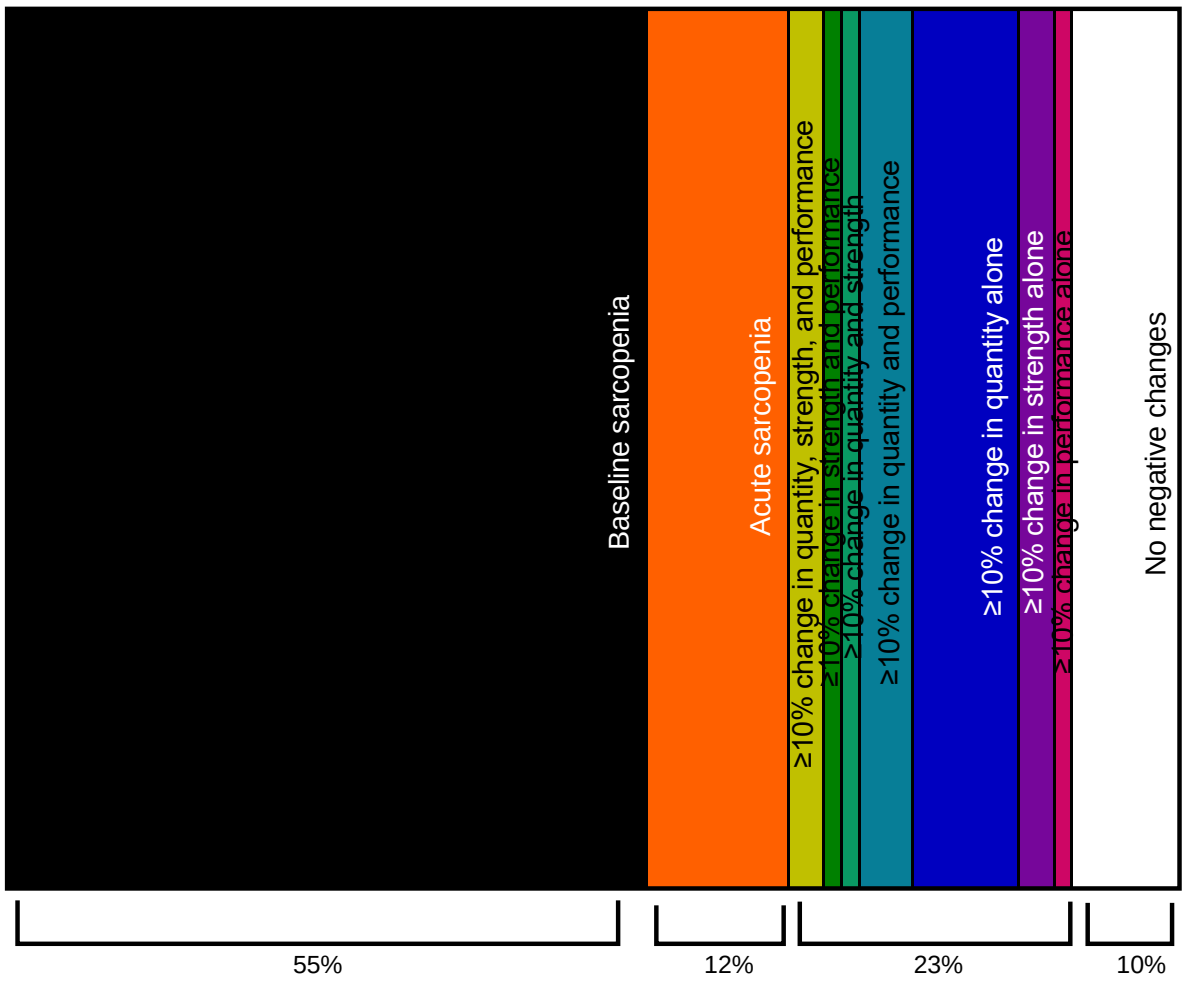


Figure 8.3-3 – Percentage of participants meeting criteria for acute sarcopenia at 7 days, or negative changes greater than or equal to 10% in those who did not meet criteria for sarcopenia

8.4 Chapter 4.2 – Supplementary information

Table 8.4-1 – Fried frailty definition.

A score of 3 or more out of 5 was considered indicative of frailty. Low physical activity definition was adapted from the Frailty Intervention Trial (Fairhall et al., 2008). All other definitions were taken from original study population definition (Fried et al., 2001).

Shrinking (Score 1 if either yes)	4.5kg weight loss over last year?		Yes	No
	Over 5% loss of previous year's body weight on examination with scales		Yes	No
Weakness (score 1 if below or equal to cut-offs)	BMI – Male	Cut-off (kg)	BMI - Female	Cut-off (kg)
	≤24	≤29	≤24	≤17
	24-26	≤30	24-26	≤17.3
	26-28	≤30	26-28	≤18
	>28	≤32	>28	≤21
Self-reported exhaustion (score 1 if answers most or all of the time to either question)	How often over the last week have you felt that the following statements were true?			
	I felt that everything I did was an effort		None of the time	
			Some of the time	
			Most of the time	
			All of the time	
	I could not get going		None of the time	
			Some of the time	
			Most of the time	
			All of the time	
Gait speed (score 1 if below or equal to cut-offs)	Height (cm) – Male	Cut-off (m/s)	Height (cm) – Female	Cut-off (m/s)
	≤173	≤0.65	≤159	≤0.65
	>173	≤0.76	>159	≤0.76
	In the last three months, have you:			

Low physical activity (score 1 if answers yes to any questions)	Performed no weight-bearing physical activity	Yes	No
	Spent more than 4 hours/ day sitting	Yes	No
	Been for a short walk once/ month or less frequently	Yes	No

Table 8.4-2 – Variables included within frailty index.

Variables were adapted from those validated within the electronic Frailty Index (eFI) (Clegg et al., 2016) for utilisation within a secondary care population.

Deficit	Definition
Activity limitation	Positive Fried physical activity score
Anaemia and haematinic deficiency	As per local reference ranges (female Hb<115, male Hb<135) or on medication for haematinic deficiency
Arthritis	Patient reported (includes osteoarthritis and inflammatory arthritis)
Atrial fibrillation	Any history – paroxysmal, temporary, or permanent
Cerebrovascular disease	Vascular dementia or stroke disease
Chronic kidney disease	eGFR <60
Diabetes mellitus	Known history/ confirmed diagnosis
Dizziness	Patient reported
Dyspnoea	Patient reported
Falls	Two or more over previous year
Foot problems	Patient reported
Fragility fracture	Previous history
Hearing impairment	Need for hearing aids
Heart failure	Known history/ confirmed diagnosis
Heart valve disease	Known history
Housebound	Lawton instrumental ADLs
Hypertension	On treatment or recorded
Presyncope/ syncope	Patient reported (altered from “hypotension” in original eFI)

Ischaemic heart disease	Known history
Memory and cognitive problems	Any cognitive spectrum disorder including mild cognitive impairment, delirium, and dementia
Osteoporosis	On treatment or known history
Parkinsonism and tremor	Includes tremor of any cause – known history or on treatment
Peptic ulcer	Known history
Peripheral vascular disease	Known history
Polypharmacy	≥5 prescribed medications
Requirement for care	Formal carers
Respiratory disease	Any history of chronic disease e.g. asthma, COPD
Skin ulcer	Present history as per Mini Nutritional Assessment (MNA) – patient reported
Sleep disturbance	Patient reported
Social vulnerability	Lives alone
Thyroid disease	Known history
Urinary or faecal incontinence	Katz ADLs
Urinary system disease	Known history
Visual impairment	Wears glasses/ visual aids or on treatment for eye condition(s)
Weight loss and anorexia	Fried weight loss OR MNA weight loss OR MNA intake decline

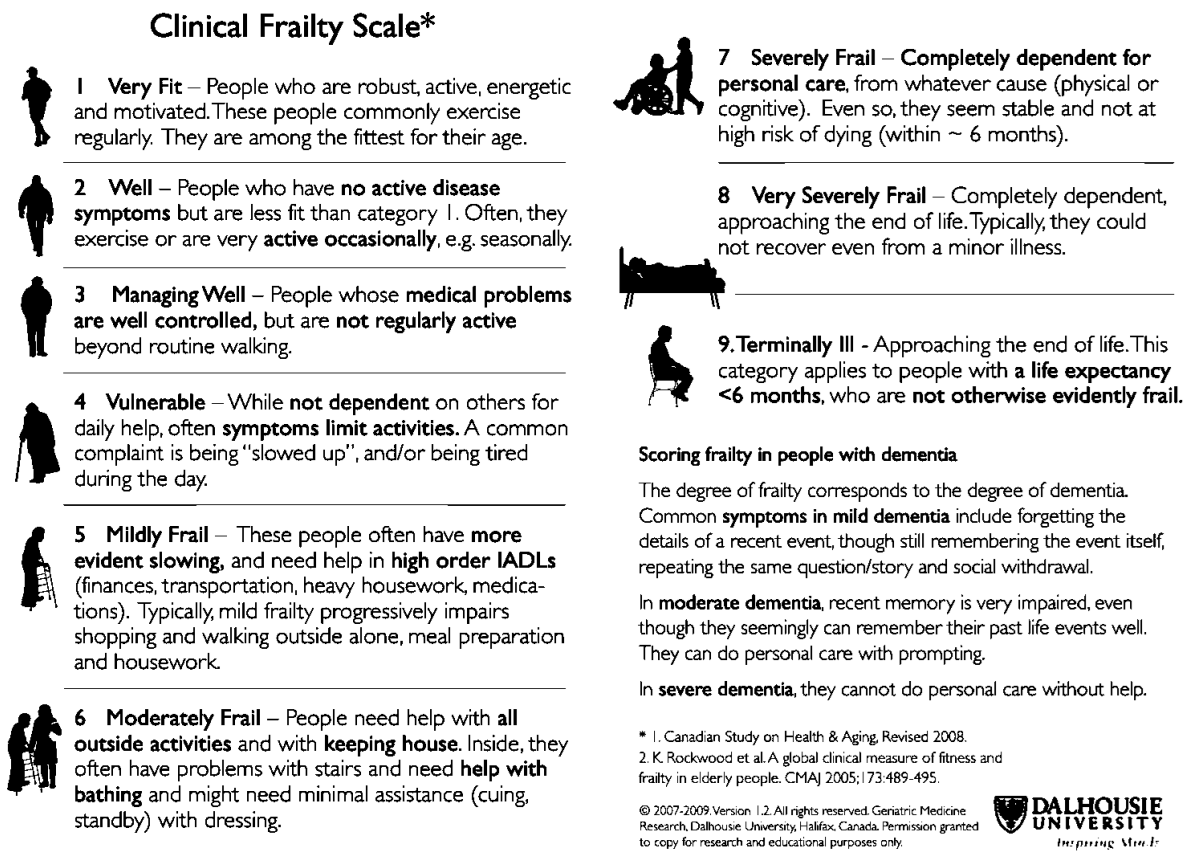


Figure 8.4-1 – Clinical Frailty Scale (2008).

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Table 8.4-3 – Cut-off values used for sarcopenia diagnosis.

Cut-off values for handgrip strength, SMMSergi, gait speed, and SPPB are taken from those recommended by the European Working Group in Older People 2 (5). Cut-off values for BATT are taken from those recommended by Wilson et al (13). BATT=Bilateral Anterior Thigh Thickness; SMMSergi=Skeletal Muscle Mass (Sergi equation).

	Male	Female
No sarcopenia	1. Handgrip strength ≥27kg	1. Handgrip strength ≥16kg
Probable sarcopenia	1. Handgrip strength <27kg 2. BATT ≥5.44cm AND SMMSergi ≥20kg	1. Handgrip strength <16kg 2. BATT ≥3.85cm AND SMMSergi ≥20kg
Definite sarcopenia, not severe	1. Handgrip strength <27kg 2. BATT <5.44cm AND/OR SMMSergi <20kg 3. Gait speed >0.8m/s AND SPPB >8	1. Handgrip strength <16kg 2. BATT <3.85cm AND/OR SMMSergi <15kg 3. Gait speed >0.8m/s AND SPPB >8
Definite sarcopenia, severity unclear	1. Handgrip strength <27kg 2. BATT <5.44cm AND/OR SMMSergi <20kg 3. Gait speed not measured AND SPPB not measured	1. Handgrip strength <16kg 2. BATT <3.85cm AND/OR SMMSergi <15kg 3. Gait speed not measured AND SPPB not measured
Definite sarcopenia, severe	1. Handgrip strength <27kg 2. BATT <5.44cm AND/OR SMMSergi <20kg 3. Gait speed ≤0.8m/s AND SPPB ≤8	1. Handgrip strength <16kg 2. BATT <3.85cm AND/OR SMMSergi <15kg 3. Gait speed ≤0.8m/s AND SPPB ≤8

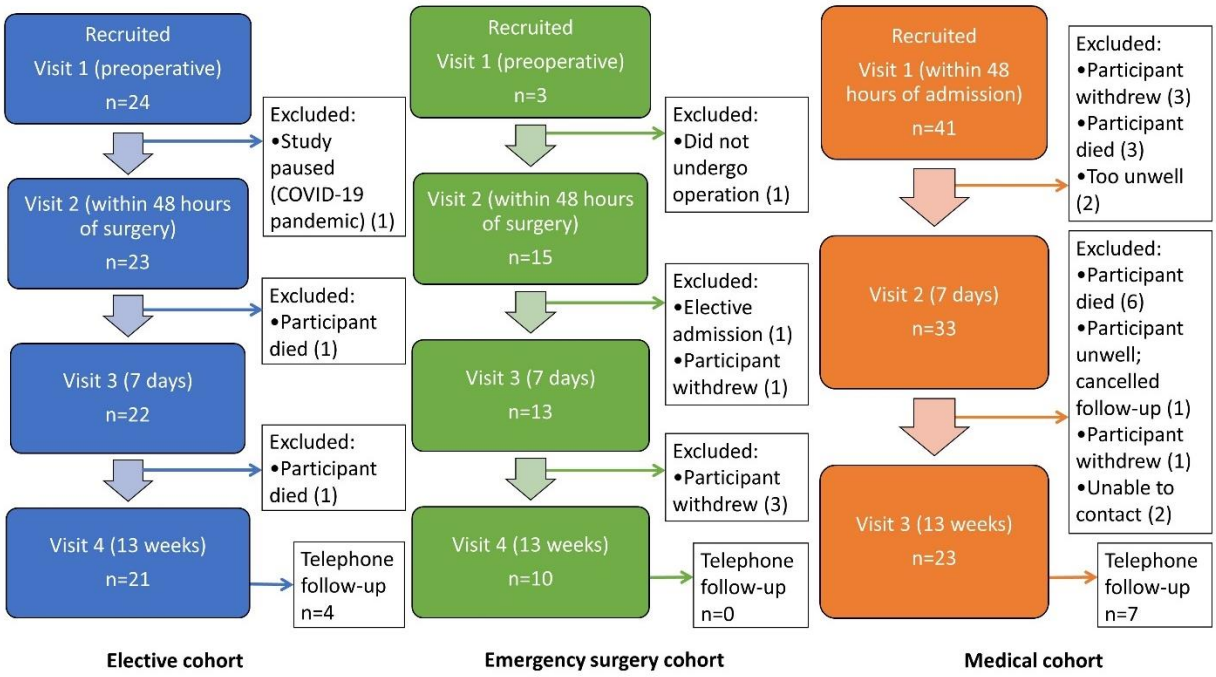


Figure 8.4-2 – Recruitment flowchart

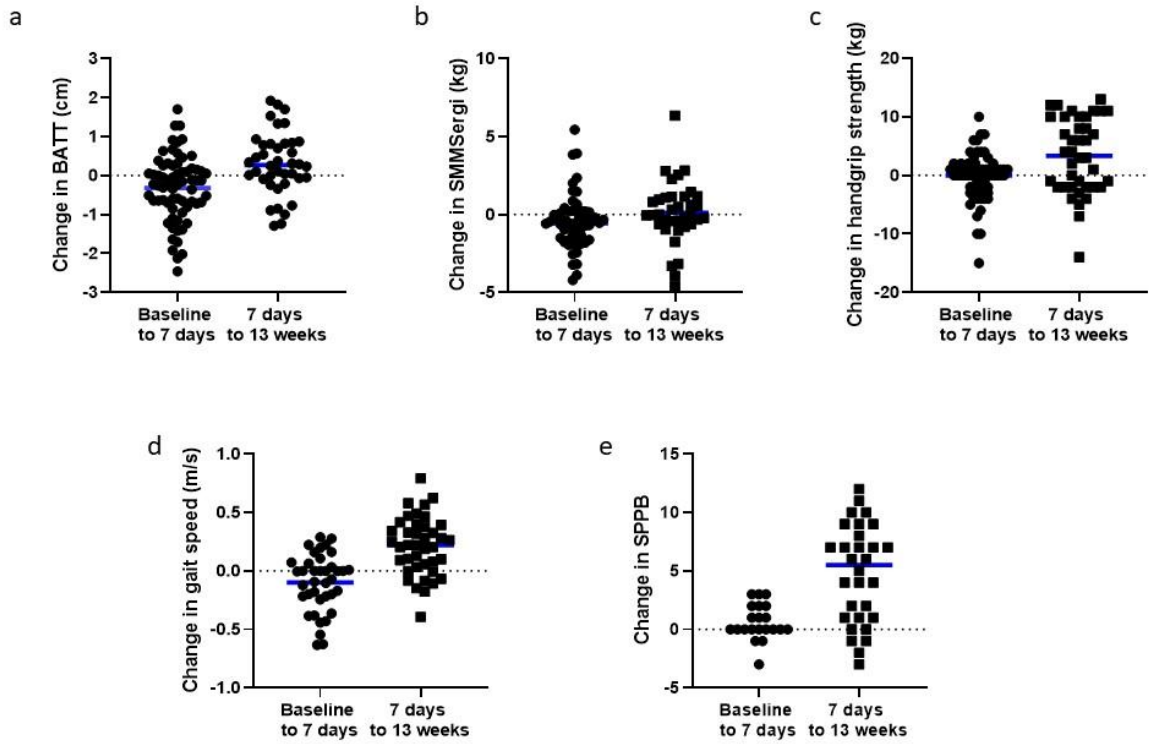


Figure 8.4-3 – Raw change scores between individual component variables between timepoints

Table 8.4-4 – Estimated marginal means for frailty indices derived from linear mixed models.

	Baseline	7 days	13 weeks	p value
Overall	0.27 (0.25 – 0.30)	0.31 (0.28 – 0.33)	0.30 (0.27 – 0.34)	0.150
Elective	0.20 (0.17 – 0.24)	0.23 (0.20 – 0.27)	0.25 (0.22 – 0.28)	0.129
Emergency	0.25 (0.18 – 0.32)	0.27 (0.19 – 0.36)	0.25 (0.13 – 0.38)	0.902
Medical	0.32 (0.29 – 0.35)	0.37 (0.33 – 0.40)	0.38 (0.32 – 0.43)	0.057

Table 8.4-5– Median Clinical Frailty Scale scores across timepoints.

Skillings-Mack and p-values are shown in the far right column. Twelve participants (2 elective, 2 emergency surgery, 8 medical) were excluded from analysis as only single baseline scores were available.

	Baseline	7 days	13 weeks	p value
Overall	4 (3 – 5)	5 (4 – 6)	4 (3 – 5)	<0.001
Elective	3 (3 – 3)	4.5 (3 – 6)	3 (3 – 4)	<0.001
Emergency	3 (3 – 4)	5 (3 – 6)	3 (3 – 5)	0.007
Medical	5 (4 – 5)	5 (4.25 – 6)	5 (4 – 6)	0.001

Table 8.4-6 – Estimated marginal means for Clinical Frailty Scale scores as derived from generalized linear mixed models.

	Baseline	7 days	13 weeks	p value
Overall	3.80 (3.54 – 4.08)	4.91 (4.59 – 5.25)	4.11 (3.74 – 4.51)	<0.001
Elective	3.00 (2.67 – 3.37)	4.55 (3.99 – 5.18)	3.38 (2.97 – 3.85)	<0.001
Emergency	3.40 (2.78 – 4.16)	4.39 (3.57 – 5.39)	3.80 (2.97 – 4.87)	0.190
Medical	4.42 (4.08 – 4.77)	5.38 (4.97 – 5.81)	4.91 (4.34 – 5.56)	0.003

Table 8.4-7 – Sarcopenia prevalence separated by severity across groups and timepoints.

		Baseline	7 days	13 weeks	p value
Overall	No sarcopenia	41.8 (33)	36.4 (24)	50.0 (20)	0.148 Simulated: 0.023
	Probable sarcopenia	7.6 (6)	4.5 (3)	5.0 (2)	
	Confirmed sarcopenia, not severe	0 (0)	0 (0)	5.0 (2)	
	Confirmed sarcopenia, severity unclear	20.3 (16)	4.5 (3)	0 (0)	
	Confirmed sarcopenia, severe	30.4 (24)	54.5 (36)	40.0 (16)	
Elective	No sarcopenia	58.3 (14)	42.9 (9)	64.7 (11)	0.396 Simulated: 0.144
	Probable sarcopenia	8.3 (2)	0 (0)	0 (0)	
	Confirmed sarcopenia, not severe	0 (0)	0 (0)	0 (0)	
	Confirmed sarcopenia, severity unclear	0 (0)	9.5 (2)	0 (0)	
	Confirmed sarcopenia, severe	33.3 (8)	47.6 (10)	35.3 (6)	
Emergency	No sarcopenia	35.7 (5)	38.5 (5)	60.0 (6)	0.117 Simulated: 0.021
	Probable sarcopenia	7.1 (1)	0 (0)	0 (0)	
	Confirmed sarcopenia, not severe	0 (0)	0 (0)	20.0 (2)	
	Confirmed sarcopenia, severity unclear	57.1 (8)	0 (0)	0 (0)	
	Confirmed sarcopenia, severe	0 (0)	61.5 (8)	20.0 (2)	

Medical	No sarcopenia	34.1 (14)	31.3 (10)	23.1 (3)	0.949 Simulated: 0.782
	Probable sarcopenia	7.3 (3)	9.4 (3)	15.4 (2)	
	Confirmed sarcopenia, not severe	0 (0)	0 (0)	0 (0)	
	Confirmed sarcopenia, severity unclear	19.5 (8)	3.1 (1)	0 (0)	
	Confirmed sarcopenia, severe	39.0 (16)	56.2 (18)	61.5 (8)	

Table 8.4-8 – Spearman's correlation between changes in frailty and sarcopenia status between baseline and 7 days

	Δ Frailty index	Δ Clinical Frailty Scale	Δ Sarcopenia status
Δ Frailty index	r_s=1.00	r_s=0.43 p<0.001*	r_s=0.09 p=0.477
Δ Clinical Frailty Scale	r_s=0.43 p<0.001*	r_s=1.00	r_s=0.16 p=0.217
Δ Sarcopenia status	r_s=0.09 p=0.477	r_s=0.16 p=0.217	r_s=1.00

Table 8.4-9 – Spearman's correlation between changes in frailty and sarcopenia status between 7 days and 13 weeks

	Δ Frailty index	Δ Clinical Frailty Scale	Δ Sarcopenia status
Δ Frailty index	r_s=1.00	r_s=0.37 p=0.018*	r_s=0.09 p=0.569
Δ Clinical Frailty Scale	r_s=0.37 p=0.018*	r_s=1.00	r_s=0.25 p=0.126
Δ Sarcopenia status	r_s=0.09 p=0.596	r_s=0.25 p=0.126	r_s=1.00

8.5 Chapter 5.1 – Supplementary information

Table 8.5-1 – Equations used in calculation of Skeletal Muscle Mass (SMM) using bioelectrical impedance analysis.

In both equations: Height in cm; Sex 1=male, 0=female; Weight in kg; Resistance in Ω; Reactance in Ω

Skeletal Muscle Parameter	Equation
SMM-Sergi	= -3.964 + [0.227 × (height^2/resistance)] + (0.095 × weight) + (1.384 × Sex) + (0.064 × reactance)
SMM-Janssen	= [(height^2/resistance) × 0.401] + (Sex × 0.3825) + (Age × -0.071) + 5.102

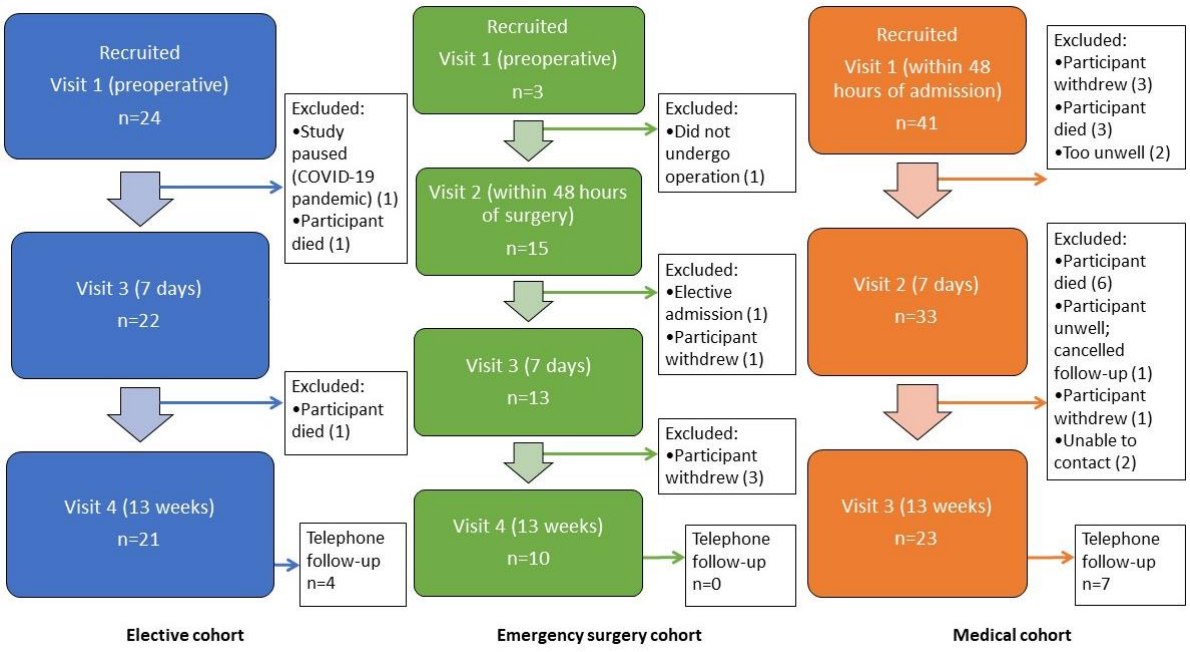


Figure 8.5-1 – Recruitment flowchart for participants included and excluded from this study

8.6 Chapter 5.2 – Supplementary information

8.6.1 Supplementary methods

8.6.1.1 Sample preparation

Blood samples were collected peripherally (or centrally if central access lines were in place as part of routine clinical care) using BD vacutainer tubes. Samples were collected in silicone coated tubes and centrifuged at 3000rpm for 10 minutes within 30-60 minutes of collection for serum separation. Samples were collected in lithium heparin tubes and centrifuged at 1600rpm for 8 minutes for plasma separation. All samples were aliquoted at time of preparation and stored at -80^{o}C prior to laboratory analysis. Samples were thawed a single time prior to analysis.

8.6.1.2 Cortisol ELISA

Cortisol was measured using Human Cortisol ELISA Kit (E-EL-0157, Elabscience). Plasma samples were diluted 1:2 using sample diluent. After preparation of reagents and standards, 50µL of standards and diluted samples were pipetted into one well each of the 96T ELISA micro-plates. Samples were pipetted in singlicate across two duplicate plates. Standards were pipetted in duplicate on both plates. Immediately, 50µL of Biotinylated Detection antibody working solution were pipetted into each well. The plates were then covered with a sealer and incubated for 45 minutes at 37^{o}C. Following this, solution was decanted from each well and 350µL of wash buffer was added to each well. Wash buffer was then decanted and the plate was tapped on absorbent paper. This wash process was repeated a further two times. Next, 100µL of Avidin conjugated to Horseradish Peroxidase (HRP) conjugate working solution was added to each well, and the plates were incubated for 30 minutes at 37^{o}C. The wash process was then repeated (three further washes), and 90µL of substrate reagent was pipetted into each well. Plates were covered with a plate sealer and incubated for 15 minutes at 37^{o}C. Finally, 50µL of stop solution were added to each well in the same order as the substrate solution. Optical density was determined immediately using a micro-plate reader set to 450nm. Sample concentrations were calculated from the standard curve using GraphPad Prism 9.2.0, using a four parameter logistic curve model. Figure 8.6-1 shows the standard curves generated.

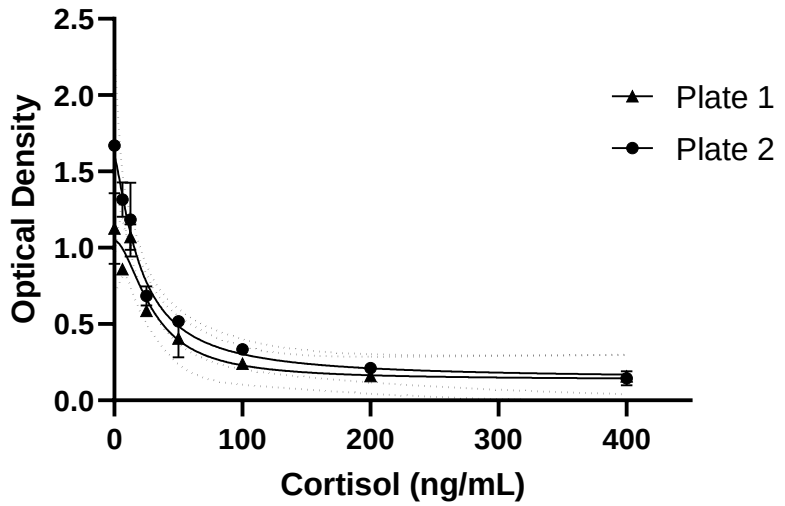


Figure 8.6-1 – Standard curves generated for cortisol ELISAs

8.6.1.3 Dehydroepiandrosterone sulfate (DHEA-s) ELISA

Dehydroepiandrosterone sulfate (DHEA-s) was measured using Human DHEA-s ELISA Kit (EH2946, FineTest, Wuhan Fine Biotech Co., Ltd.). Plasma samples were diluted 1:2 using sample dilution buffer. Before adding standards and samples, the 96T ELISA micro-plates were washed twice by pipetting 350µL of wash buffer into each well and decanting. After preparation of reagents and standards, 50µL of standards and diluted samples were pipetted into one well each of the plates. Samples were pipetted in singlicate across two duplicate plates. Standards were pipetted in duplicate on both plates. Immediately, 50µL of Biotin-labelled antibody working solution were pipetted into each well. The plates were then covered with a sealer and incubated for 45 minutes at 37°C. Following this, the wash process was repeated three times. Next, 100µL of HRP-Streptavidin conjugate working solution was added to each well, and the plates were incubated for 30 minutes at 37°C. The wash process was then repeated (five further washes), and 90µL of substrate reagent was pipetted into each well. Plates were covered with a plate sealer and incubated for 15 minutes at 37°C. Finally, 50µL of stop solution were added to each well in the same order as the substrate solution. Optical density was determined immediately using a micro-plate reader set to 450nm. Sample concentrations were calculated from the standard curve using GraphPad Prism 9.2.0, using a four parameter logistic curve model. Figure 8.6-2 shows the standard curves generated.

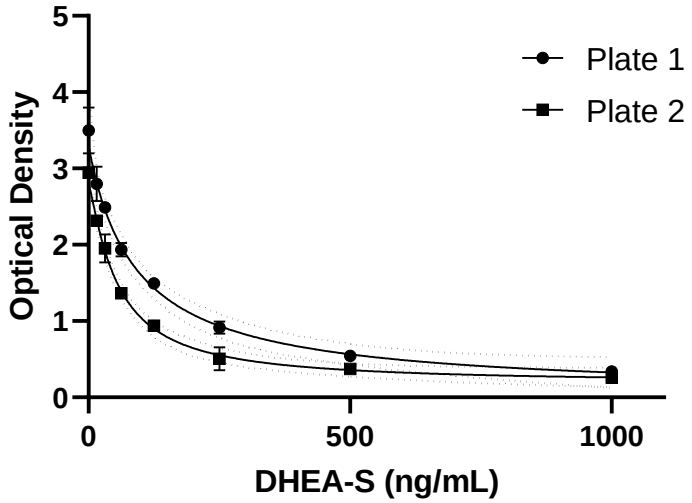


Figure 8.6-2 – Standard curves generated for DHEA-s ELISAs

8.6.1.4 hsCRP ELISA

High sensitivity C-Reactive Protein (hsCRP) was measured using Human hsCRP ELISA Kit (HK369, HycultBiotech). Standards were diluted 1:100 and serum samples were diluted 1:1000 using sample dilution buffer. Subsequently, 100µL of diluted standards and samples were pipetted into each well of the 96T micro-plates. Samples were pipetted in singlicate across two duplicate plates. Standards were pipetted in duplicate on both plates. The plates were then covered and incubated at room temperature for 30 minutes. Following this, solution was decanted from each well and 350µL of wash buffer was added to each well. Wash buffer was then decanted and the plate was tapped on absorbent paper. This wash process was repeated a further two times. Next, 100µL of conjugate solution was pipetted into each well. Plates were then covered and incubated at room temperature for 30 minutes, following which the washing procedure was repeated. Next,100µL of Chromagen solution was added to each well, then the plates were recovered and incubated for 10 minutes at room temperature. Finally, 50µL of stop solution were added to each well in the same order as the substrate solution. Optical density was determined immediately using a micro-plate reader set to 450nm. Sample concentrations were calculated from the standard curve using GraphPad Prism 9.2.0, using a linear model. Figure 8.6-3 shows the standard curves generated.

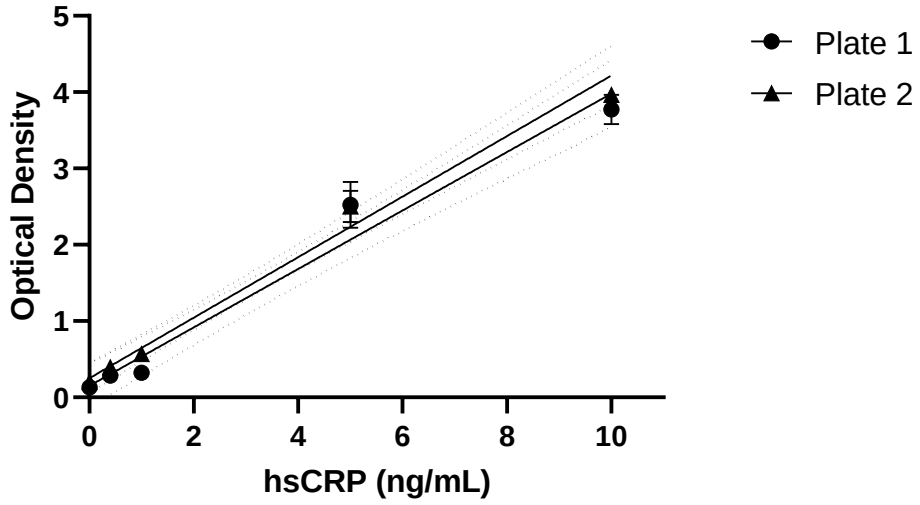


Figure 8.6-3 – Standard curves generated for hsCRP ELISAs

8.6.2 Growth Hormone (GH) ELISA

Growth Hormone (GH) was measured using Human Growth Hormone sandwich ELISA kit (KE00167, Proteintech). Serum samples were diluted 1:2 using sample diluent PT 1-em. After preparation of reagents and standards, 100µL of standards and diluted samples were pipetted into one well each of the 96T ELISA micro-plates. Samples were pipetted in singlicate across two duplicate plates. Standards were pipetted in duplicate on both plates. The plates were then covered with a sealer and incubated for 120 minutes at 37°C. Following this, solution was decanted from each well and 350µL of wash buffer was added to each well. Wash buffer was then decanted and the plate was tapped on absorbent paper. This wash process was repeated a further three times. Next, 100µL of diluent antibody solution was added to each well, and the plates were incubated for 60 minutes at 37°C. The wash process was then repeated (four further washes), and 100µL of diluent HRP solution was pipetted into each well. Plates were covered with a plate sealer and incubated for 40 minutes at 37°C, and the wash process (four further washes) was repeated again. Following this, 100µL of substrate was added to each well and the plate was incubated for a further 15 minutes in the dark at 37°C. Finally, 100µL of stop solution was added to each well in the same order as the substrate solution. Optical density was determined immediately using a micro-plate reader set to 450nm. Sample concentrations were calculated from the standard curve using GraphPad Prism 9.2.0, using a four parameter logistic curve model. Figure 8.6-4 shows the standard curves generated.

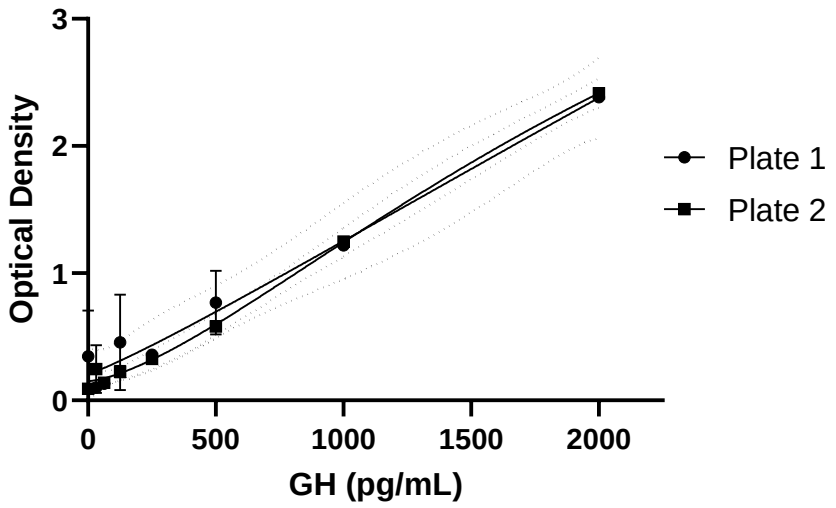


Figure 8.6-4 – Standard curves generated for GH ELISAs

8.6.3 Insulin-like Growth Factor 1 (IGF-1) ELISA

Insulin-like Growth Factor 1 (IGF-1) was measured using Human IGF-1 ELISA Kit (ELH-IGF1, RayBiotech). Serum samples were diluted 1:2 using diluent buffer. After preparation of reagents and standards, 100µL of standards and diluted samples were pipetted into one well each of the 96T ELISA micro-plates. Samples were pipetted in singlicate across two duplicate plates. Standards were pipetted in duplicate on both plates. The plates were then covered with a sealer and incubated for 150 minutes at room temperature with gentle shaking. Following this, solution was decanted from each well and 300µL of wash buffer was added to each well. Wash buffer was then decanted and the plate was tapped on absorbent paper. This wash process was repeated a further three times. Next, 100µL of biotinylated antibody solution was added to each well, and the plates were incubated for 60 minutes at room temperature with gentle shaking. The wash process was then repeated (four further washes), and 100µL of streptavidin solution was pipetted into each well. Plates were covered with a plate sealer and incubated for 45 minutes at room temperature with gentle shaking, and the wash process (four further washes) was repeated again. Following this, 100µL of substrate reagent was added to each well and the plate was incubated for a further 30 minutes in the dark at room temperature with gentle shaking. Finally, 50µL of stop solution was added to each well in the same order as the substrate solution. Optical density was determined immediately using a micro-plate reader set to 450nm. Sample concentrations were calculated from the standard curve using GraphPad Prism 9.2.0, using a four parameter logistic curve model. Figure 8.6-5 shows the standard curves generated.

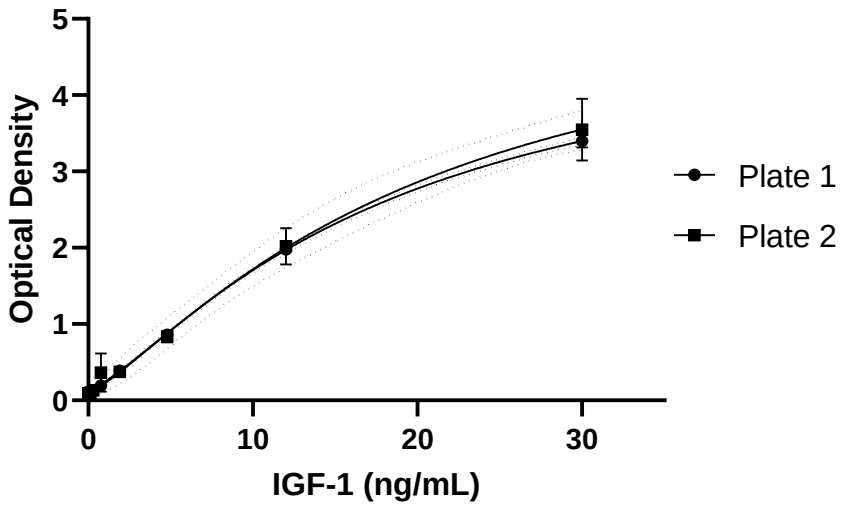


Figure 8.6-5 - Standard curves generated for IGF-1 ELISAs

8.6.4 Myostatin ELISA

Myostatin was measured using Human Myostatin ELISA Kit (DL-MSTN-Hu, Dldevelop). Serum samples were diluted 1:2 using diluent buffer. After preparation of reagents and standards, 100µL of standards and diluted samples were pipetted into one well each of the 96T ELISA micro-plates. Samples were pipetted in singlicate across two duplicate plates. Standards were pipetted in duplicate on both plates. The plates were then covered with a sealer and incubated for 120 minutes at 37°C. Following this, solution was decanted from each well. Next, 100µL of Detection Reagent A working solution was added to each well, and the plates were covered and incubated for 60 minutes at 37°C. Solution was then decanted from each well and 300µL of wash buffer was added to each well. Wash buffer was then decanted and the plate was tapped on absorbent paper. This wash process was repeated a further two times. Next, 100µL of Detection Reagent B working solution was pipetted into each well. Plates were covered with a plate sealer and incubated for 60 minutes at 37°C, and the wash process (five further washes) was repeated again. Following this, 90µL of substrate solution was added to each well and the plate was covered and incubated for a further 15 minutes at 37°C. Finally, 50µL of stop solution was added to each well in the same order as the substrate solution. Optical density was determined immediately using a micro-plate reader set to 450nm. Sample concentrations were calculated from the standard curve using GraphPad Prism 9.2.0, using a four parameter logistic curve model. Figure 8.6-6 shows the standard curves generated.

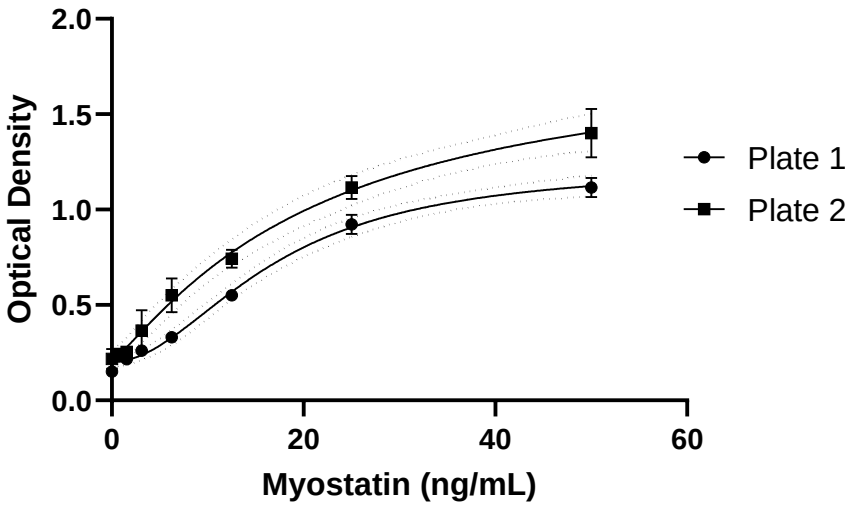


Figure 8.6-6 – Standard curves generated for Myostatin ELISAs

8.6.5 Vitamin D ELISA

Total 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3 (total 25-OH Vitamin D) was measured using Total 25-OH Vitamin D ELISA Kit (80987, Crystal Chem). The working conjugate was prepared prior to pipetting of samples and standards. Samples were used neat and undiluted in this experiment; 25µL of standards and samples were pipetted into each well of the 96T micro-plates. Samples were pipetted in singlicate across two duplicate plates. Standards were pipetted in duplicate on both plates. Next, 150µL of incubation buffer was added to each wells. The plates were then covered and incubated at room temperature for 60 minutes. Following this, solution was aspirated from wells and wells were washing with wash buffer three times using an automated plate washer (R&D systems). Following this, 150µL of working conjugate solution was pipetted into each well. Plates were then covered and incubated at room temperature for 30 minutes, following which the washing procedure was repeated. Next,150µL of substrate solution was added to each well, then the plates were recovered and incubated for 15 minutes at room temperature. Finally, 50µL of stop solution were added to each well in the same order as the substrate solution. Optical density was determined immediately using a micro-plate reader set to 450nm. Sample concentrations were calculated from the standard curve using GraphPad Prism 9.2.0, using a linear model. Figure 8.6-7 shows the standard curves generated.

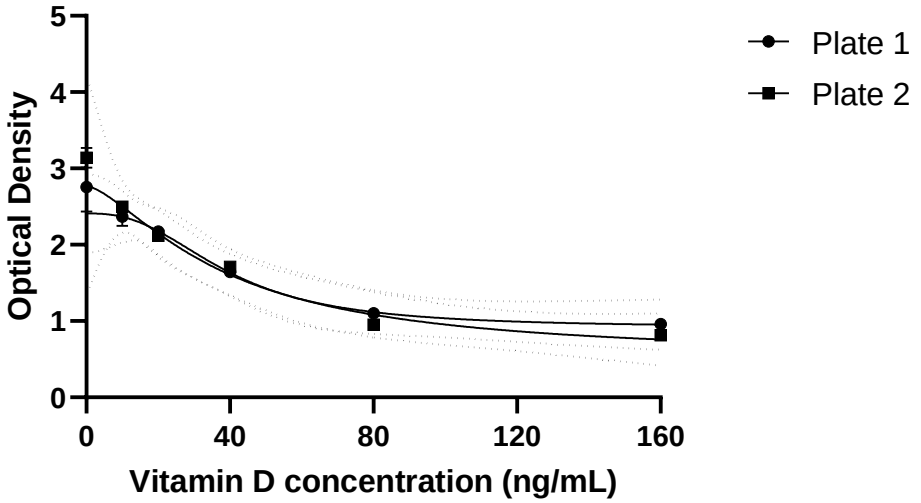
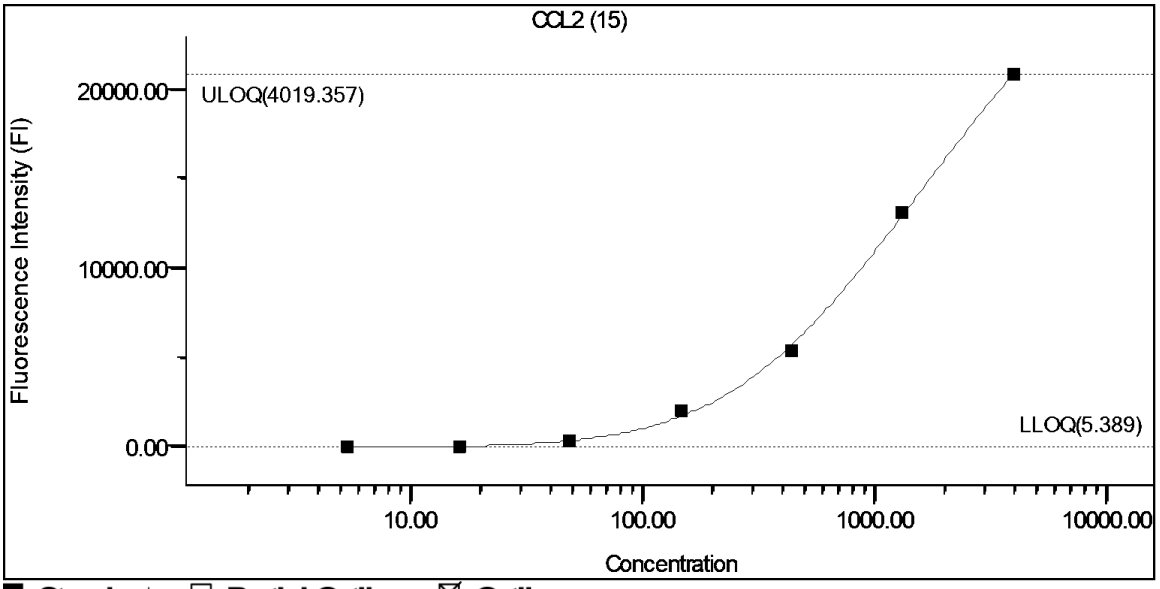


Figure 8.6-7 – Standard curves generated for 25-OH Vitamin D ELISA

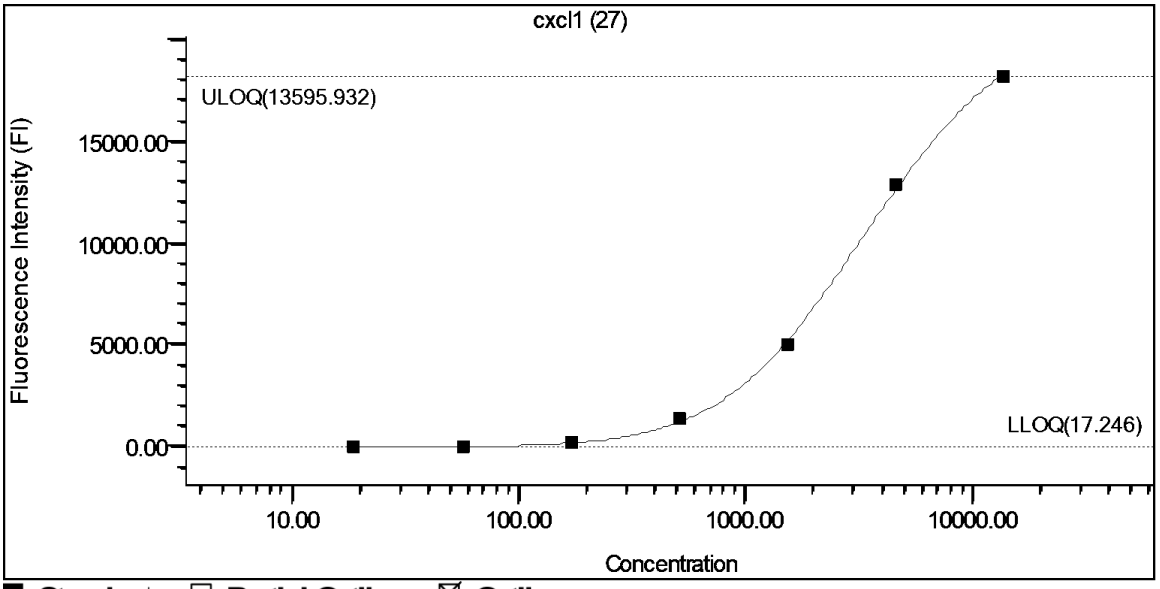
8.6.6 Human XL cytokine Luminex assay

CCL2/JE/MCP-1, CXCL1/GRO alpha/KC/CINC-1, Flt-2 Ligand/FLT3L, IL-1 alpha/IL-1F1, IL-4, IL-7, IL-10, TNF-alpha, CCL3/MIP-1 alpha, CXCL10/IP-10/CRG-2, IFN-gamma, IL-1 beta/IL-1F2, IL-6, IL-8/CXCL8, IL-15, and VEGF were measured using Human XL Cytokine Premixed Luminex Performance Assay Kit (1621325, R&D systems, Bio-techne). Samples were diluted 1:2 with calibrator diluent RD-65. After preparation of reagents and standards, 50µL of standards and diluted samples was pipetted into each well of the 96T micro-titre plates. Samples were pipetted in singlicate across two duplicate plates. Standards were pipetted in duplicate on both plates. Subsequently, 50µL of diluted microparticle cocktail was added to each well of the micro-titre plates. Plates were covered and incubated for 120minutes at room temperature on a horizontal orbital plate shaker set at 800rpm. Following this, solution was aspirated from the wells and the plates were washed with wash buffer three times using an electronic plate washer with a magnetic plate holder (R&D systems). Next, 50µL of diluted Biotin-Antibody cocktail was added to all wells, the plates were covered with a sealer and incubated for 60 minutes at room temperature on the shaker set at 800rpm. The wash process was then repeated. Following this 50µL of diluted Streptavidin-PE was pipetted to all wells. Plates were again covered with a sealer and incubated for 30minutes at room temperature on the shaker at 800rpm. The wash process was again repeated after this. Finally, the microparticles were resuspended by adding 100µL of wash buffer to each well. Plates were incubated on the shaker set at 800rpm for two minutes. Plates were read using a Bio-Rad analyser. Standard curves generated from the assay are shown in Figure 8.6-8, Figure 8.6-9, Figure 8.6-10, Figure 8.6-11, Figure 8.6-12, Figure 8.6-13, Figure 8.6-14, Figure 8.6-15, Figure 8.6-16, Figure 8.6-17, Figure 8.6-18, Figure 8.6-19, Figure 8.6-20, Figure 8.6-21, and Figure 8.6-22.



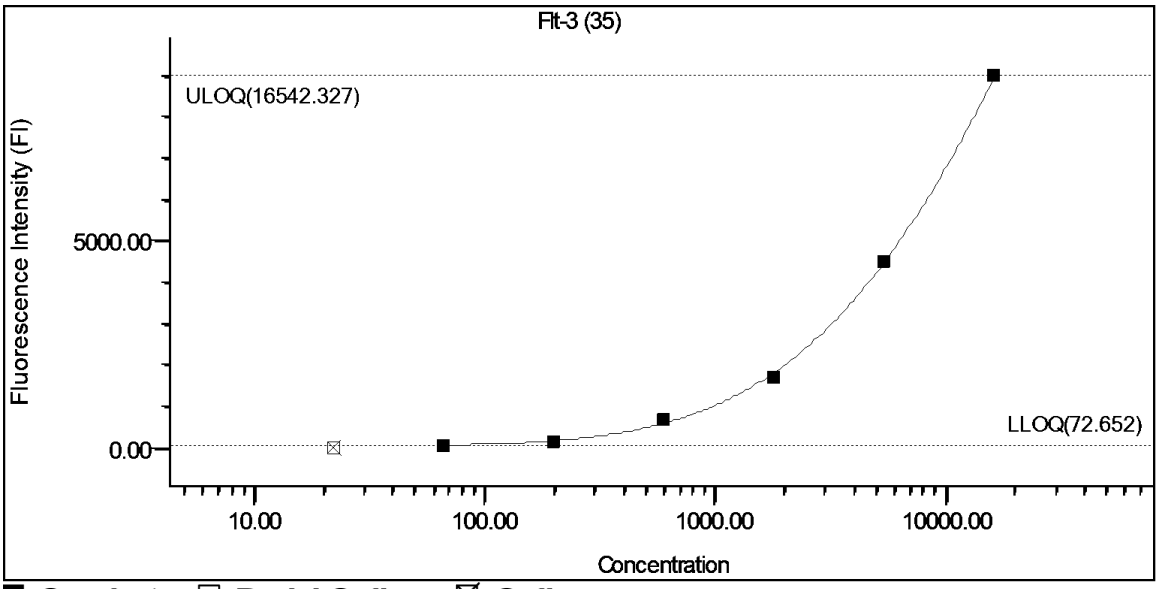
Regression Type: Logistic - 5PL
Std. Curve: FI = -2.49099 + (33105 + 2.49099) / ((1 + (Conc / 488.713)^-0.737658))^2.39775
FitProb. = 0.0009, ResVar. = 6.9698

Figure 8.6-8 – Standard curves for CCL2 generated from Luminex assays



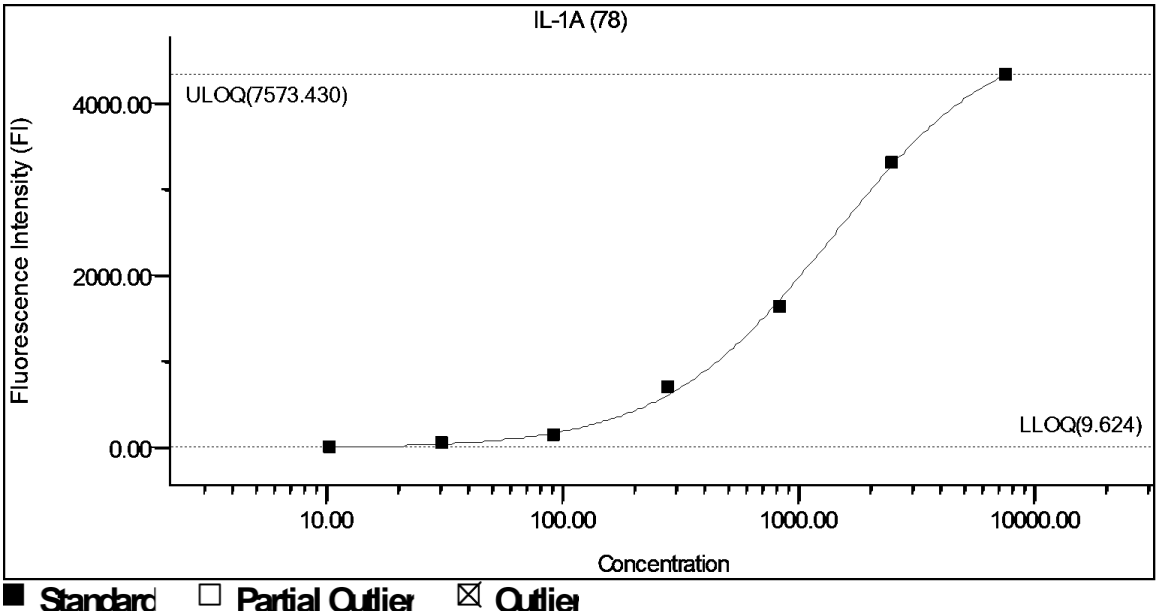
Regression Type: Logistic - 5PL
Std. Curve: FI = -0.469562 + (21682 + 0.469562) / ((1 + (Conc / 2320.7)^-1.18535))^1.48024
FitProb. = 0.0068, ResVar. = 4.9855

Figure 8.6-9 – Standard curves for CXCL1 generated from Luminex assays



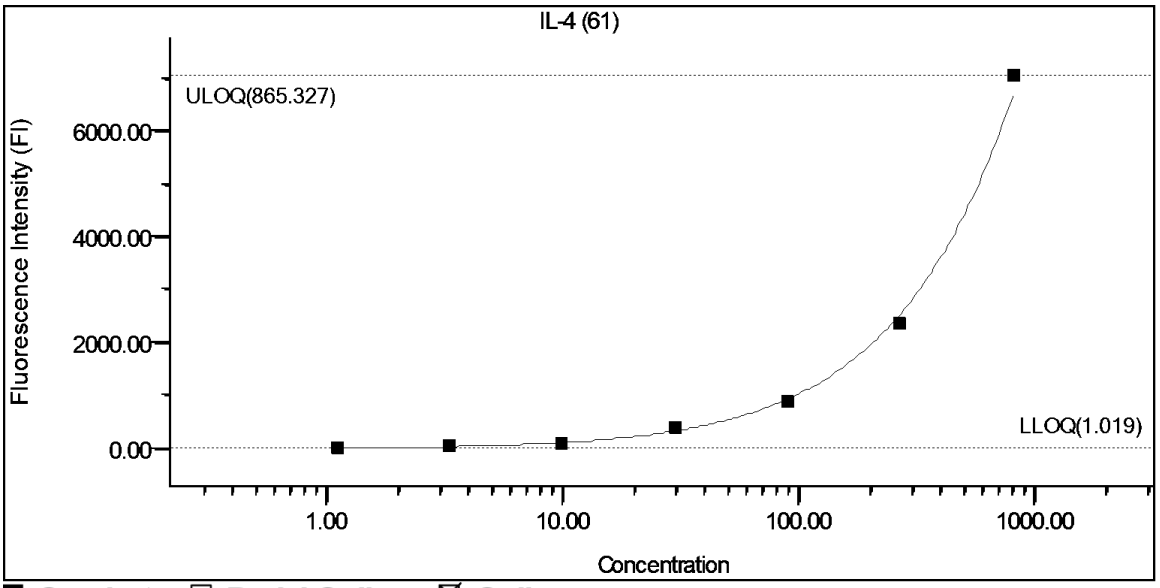
Regression Type: Logistic - 5PL
Std. Curve: FI = 73.5957 + (59569.4 - 73.5957) / ((1 + (Conc / 119.769)^-0.318332))^10
FitProb. = 0.0059, ResVar. = 7.5801

Figure 8.6-10 – Standard curves for Flt-2 generated from Luminex assays



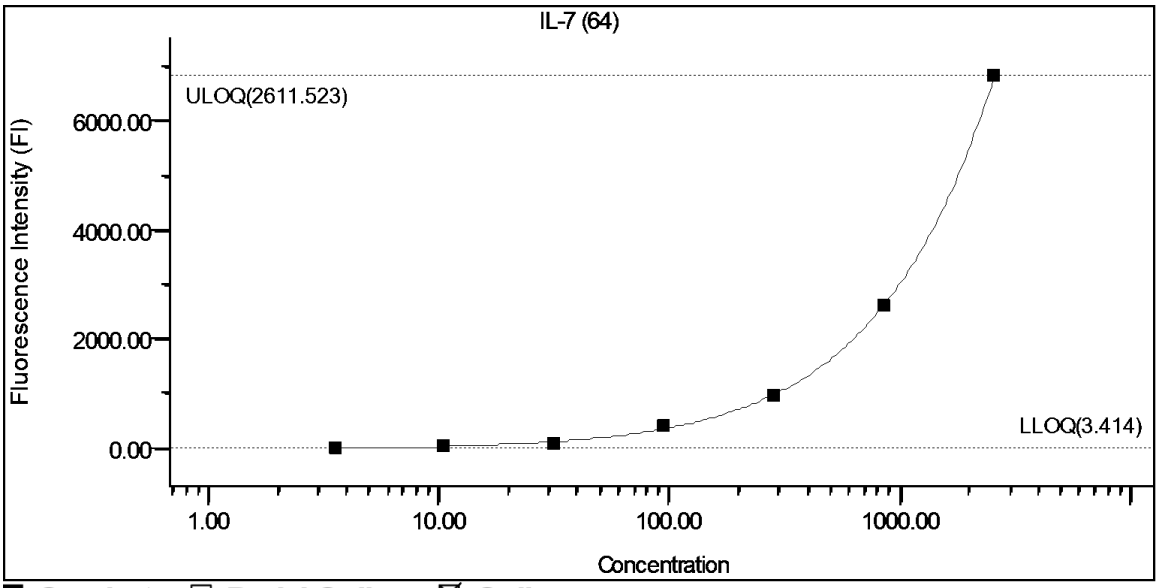
Regression Type: Logistic - 5PL
Std. Curve: FI = -3.32524 + (4943.85 + 3.32524) / ((1 + (Conc / 1374.02)^-1.19575))^1.01706
FitProb. = 0.0000, ResVar. = 14.2879

Figure 8.6-11 – Standard curves for IL-1A generated from Luminex assays



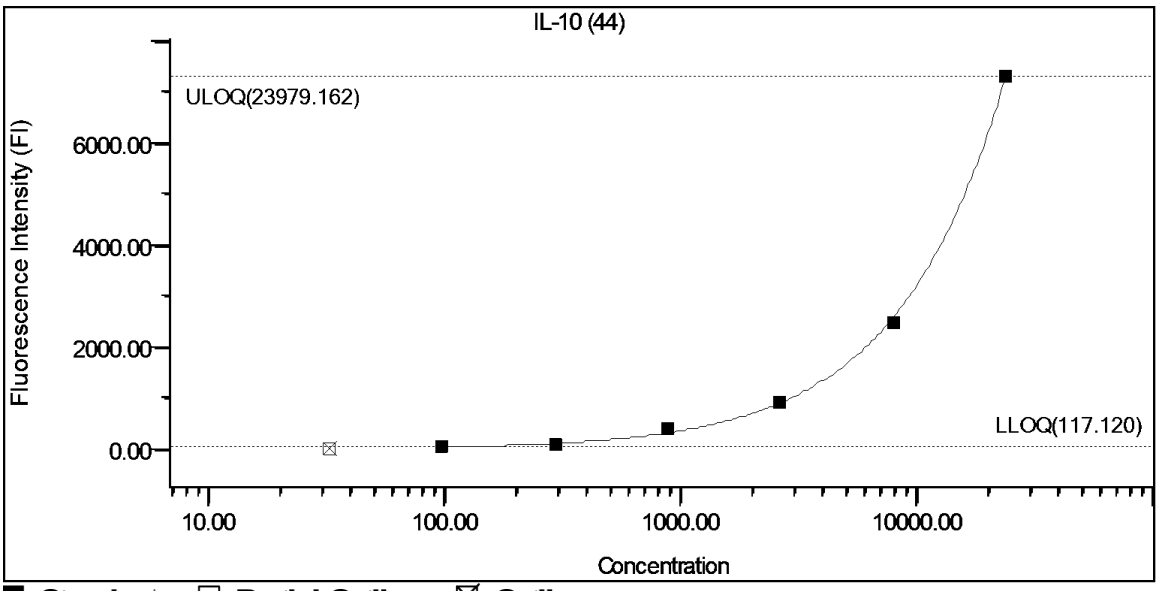
Regression Type: Logistic - 5PL
Std. Curve: FI = -1.46266 + (7.0975E+007 + 1.46266) / ((1 + (Conc / 2.40272E+006)^-0.199401))^5.21004
FitProb. = 0.0000, ResVar. = 11.4093

Figure 8.6-12 – Standard curves for IL-4 generated from Luminex assays



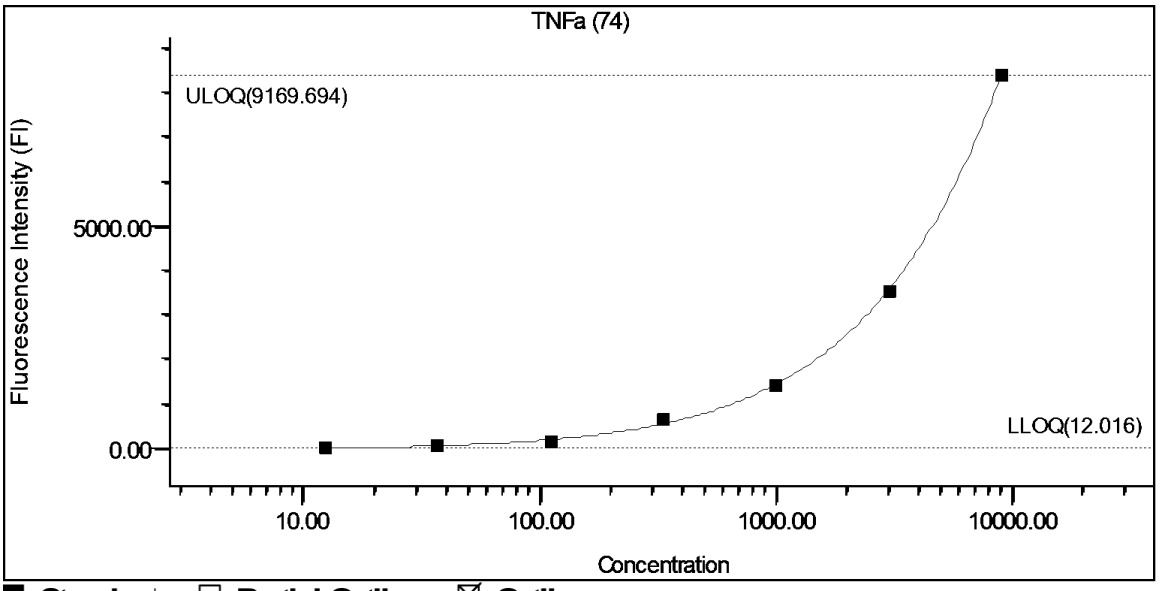
Regression Type: Logistic - 5PL
Std. Curve: FI = -7.315 + (1.15814E+006 + 7.315) / ((1 + (Conc / 642946)^-0.486986))^1.86579
FitProb. = 0.0000, ResVar. = 11.3016

Figure 8.6-13 – Standard curves for IL-7 generated from Luminex assays



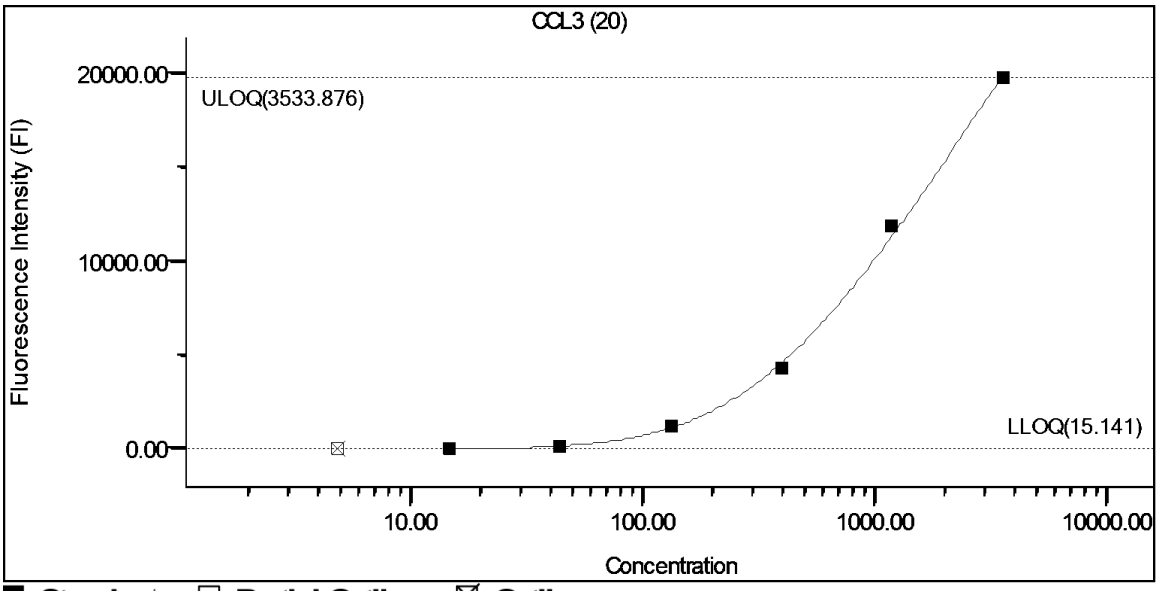
Regression Type: Logistic - 5PL
Std. Curve: FI = 12.7987 + (414317 - 12.7987) / ((1 + (Conc / 1.69335E+006)^-2.34548))^0.404138
FitProb. = 0.0000, ResVar. = 17.2252

Figure 8.6-14 – Standard curves for IL-10 generated from Luminex assays



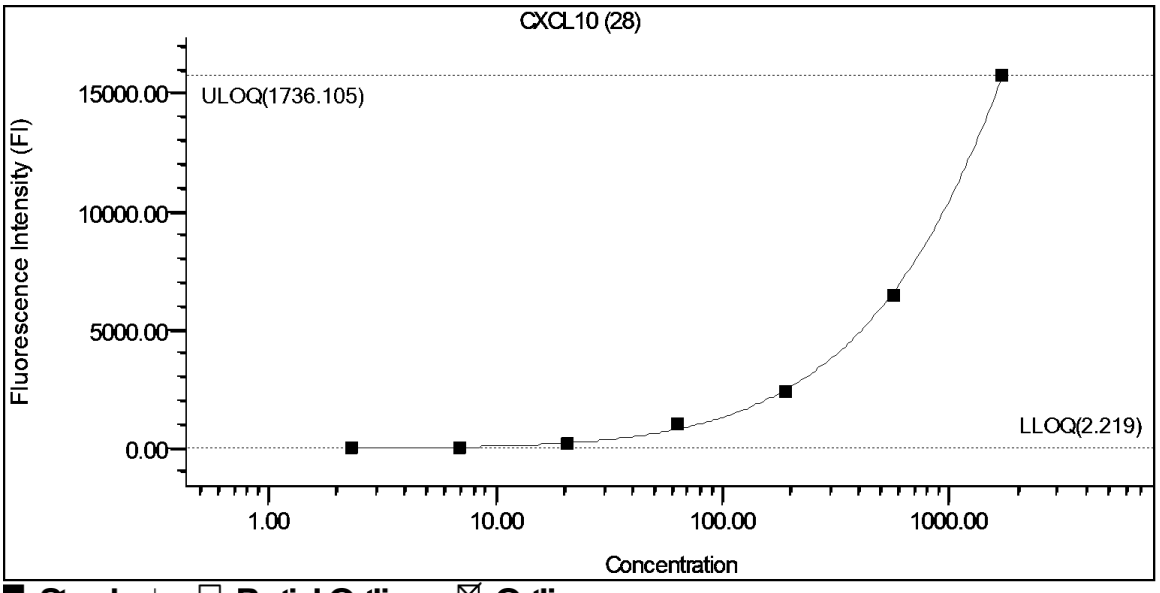
Regression Type: Logistic - 5PL
Std. Curve: FI = -6.75577 + (637574 + 6.75577) / ((1 + (Conc / 621988)^-0.363886))^2.50028
FitProb. = 0.0003, ResVar. = 8.1017

Figure 8.6-15 – Standard curves for TNF-alpha generated from Luminex assays



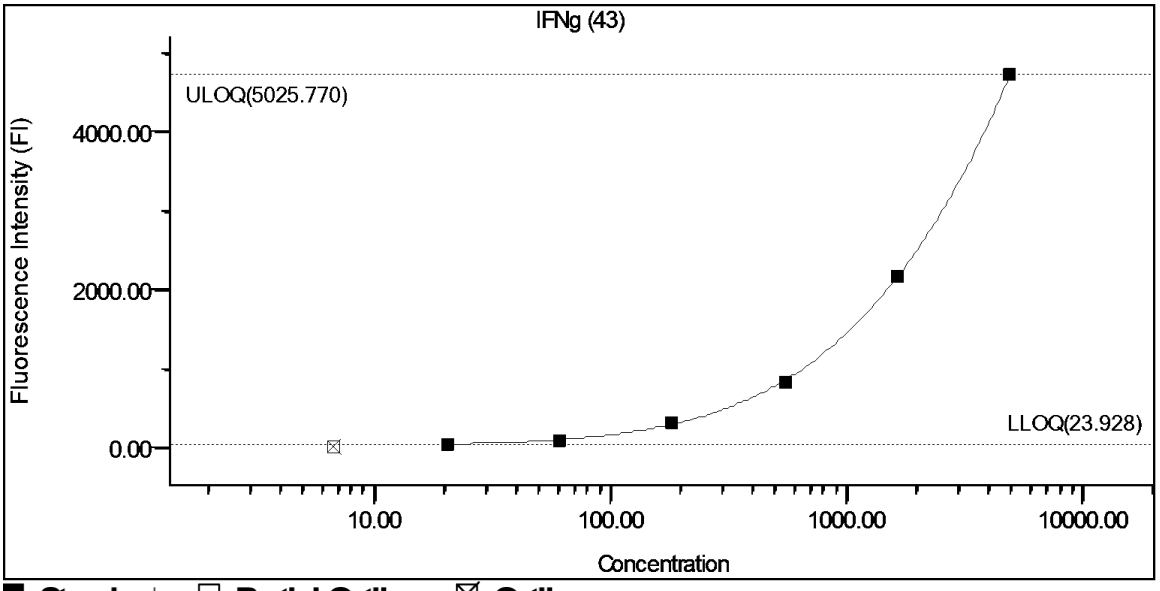
Regression Type: Logistic - 5PL
Std. Curve: FI = 25.3359 + (44786 - 25.3359) / ((1 + (Conc / 27.1937)^-0.505754))^10
FitProb. = 0.0219, ResVar. = 5.2531

Figure 8.6-16 – Standard curves for CCL3 generated from Luminex assays



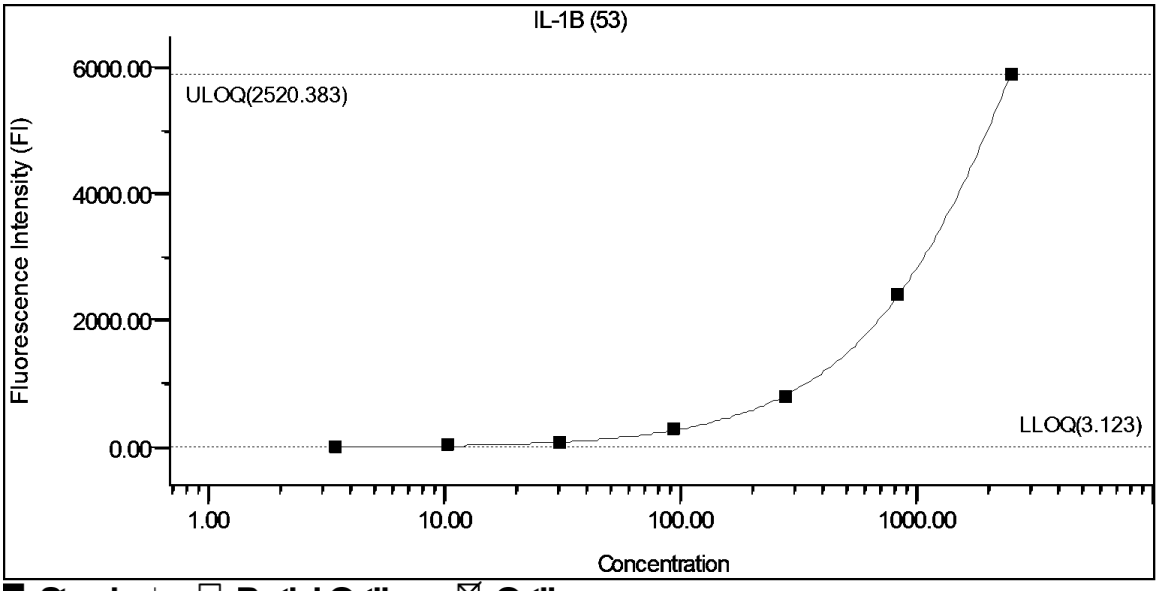
Regression Type: Logistic - 5PL
Std. Curve: FI = 2.15748 + (714980 - 2.15748) / ((1 + (Conc / 829.647)^-0.254568))^6.31294
FitProb. = 0.0000, ResVar. = 17.2950

Figure 8.6-17 – Standard curves for CXCL10 generated from Luminex assays



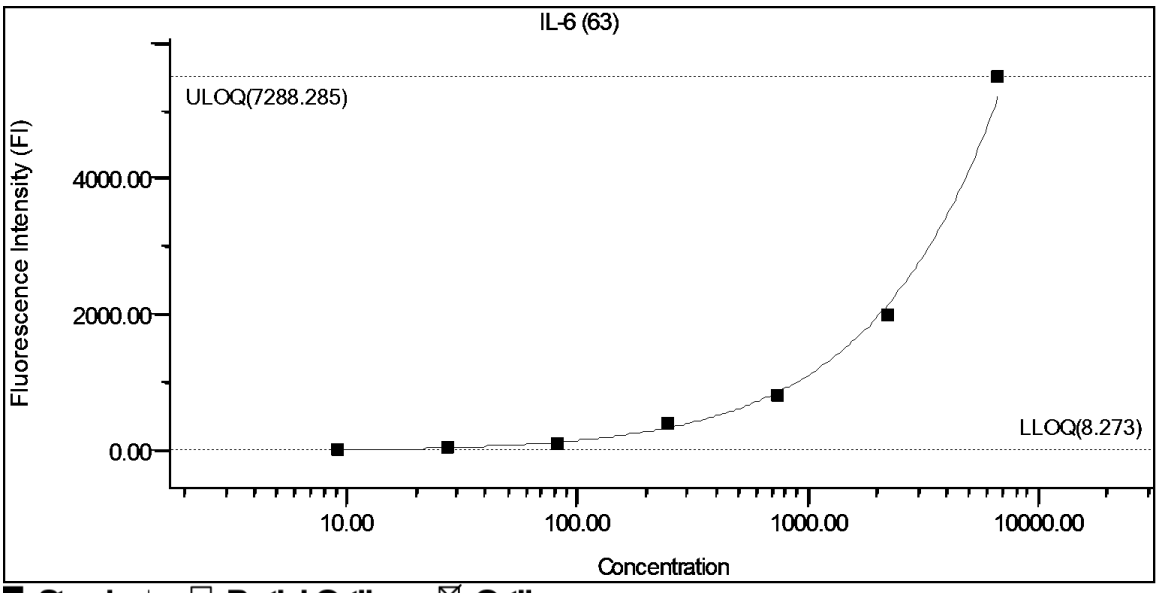
Regression Type: Logistic - 5PL
Std. Curve: FI = 43.2908 + (89218.8 - 43.2908) / ((1 + (Conc / 71.9159)^-0.252547))^10
FitProb. = 0.0556, ResVar. = 3.6646

Figure 8.6-18 – Standard curves for interferon gamma generated from Luminex assays



Regression Type: Logistic - 5PL
Std. Curve: FI = -0.168151 + (13726.9 + 0.168151) / ((1 + (Conc / 4198.87)^-1.33084))^0.771539
FitProb. = 0.0005, ResVar. = 7.6347

Figure 8.6-19 – Standard curves for IL-1beta generated from Luminex assays

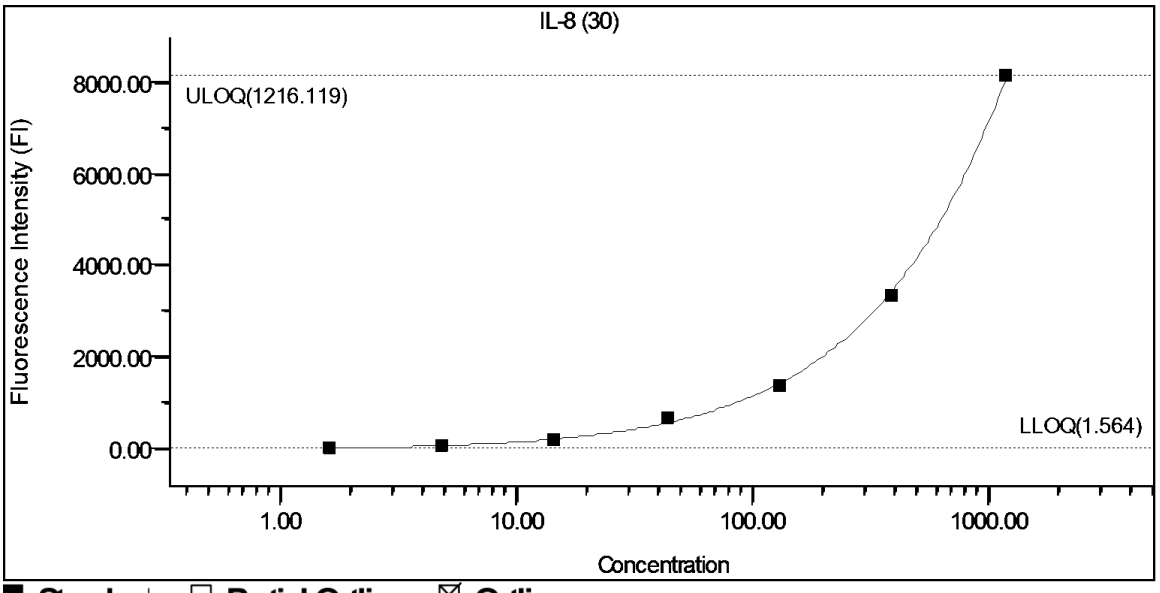


Regression Type: Logistic - 5PL

Std. Curve: FI = -5.65194 + (5.5565E+007 + 5.65194) / ((1 + (Conc / 5.62996E+007)^-0.188462))^4.96196

FitProb. = 0.0000, ResVar. = 10.1381

Figure 8.6-20 – Standard curves for IL-6 generated from Luminex assays

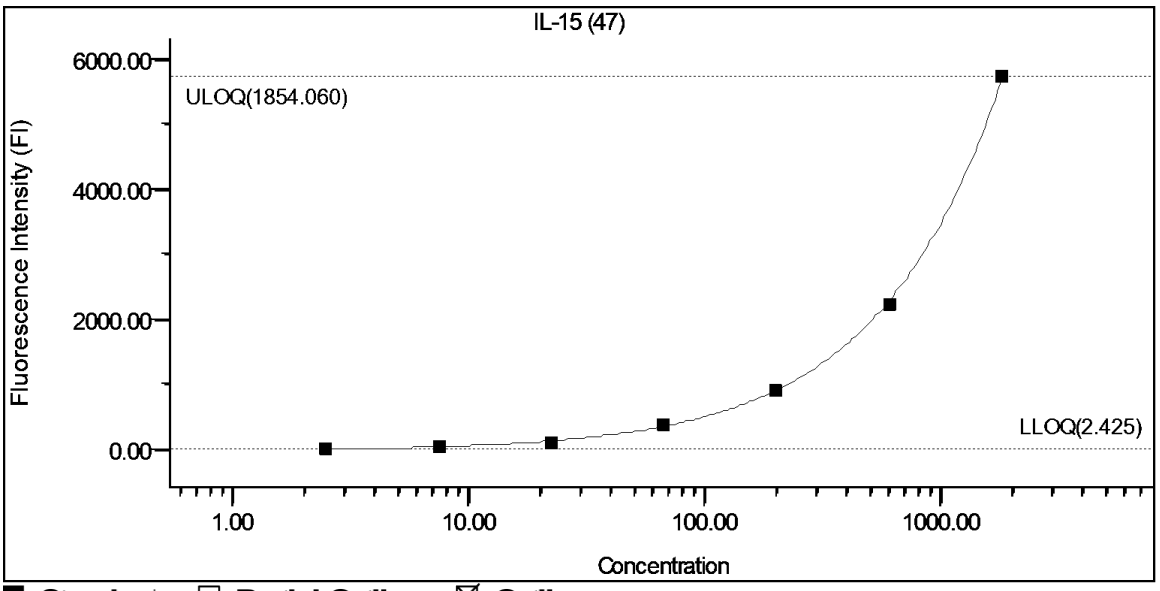


Regression Type: Logistic - 5PL

Std. Curve: FI = -10.9992 + (8.19625E+007 + 10.9992) / ((1 + (Conc / 879301)^-0.143614))^7.23118

FitProb. = 0.0001, ResVar. = 9.4438

Figure 8.6-21 – Standard curves for IL-8 generated from Luminex assays

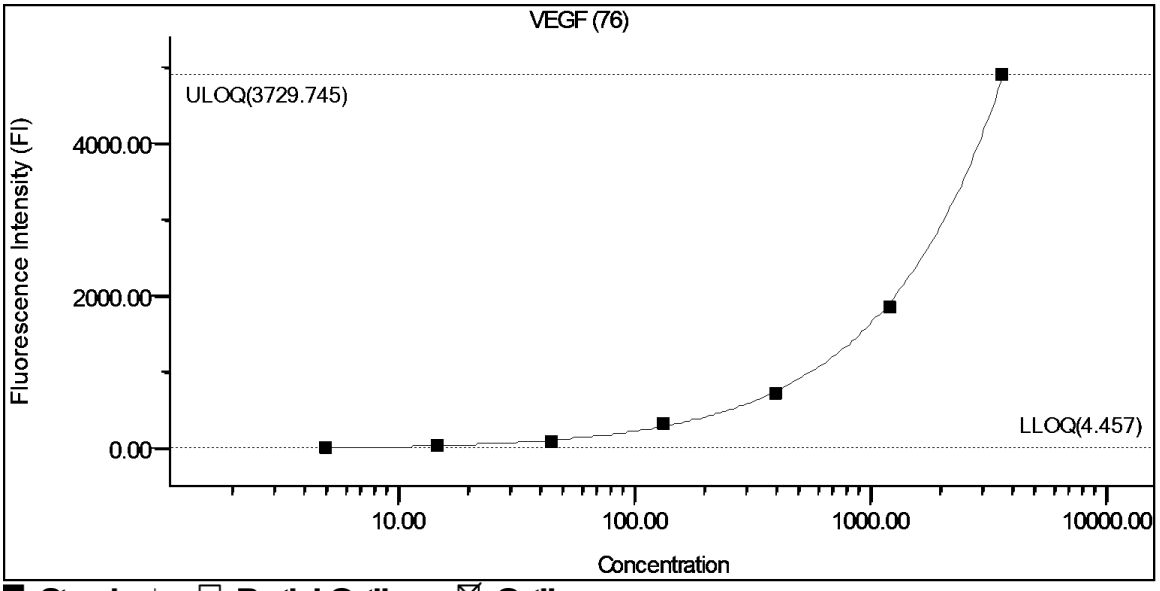


Regression Type: Logistic - 5PL

Std. Curve: FI = -10.9228 + (197908 + 10.9228) / ((1 + (Conc / 136327)^-2.30963))^0.356287

FitProb. = 0.0014, ResVar. = 6.5650

Figure 8.6-22 – Standard curves for IL-15 generated from Luminex assays



Regression Type: Logistic - 5PL

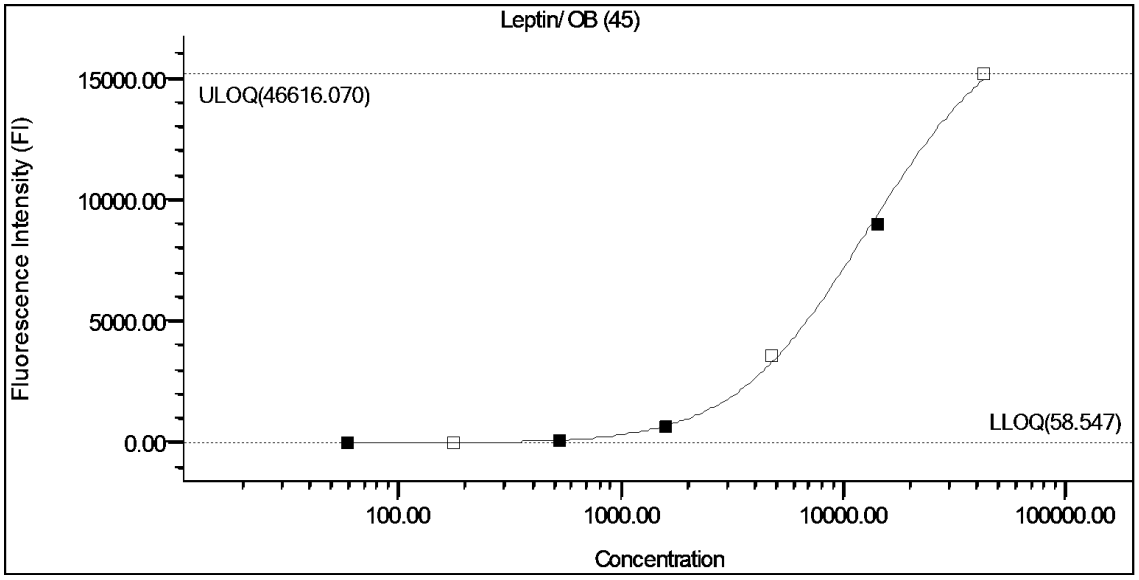
Std. Curve: FI = -3.78215 + (36903.4 + 3.78215) / ((1 + (Conc / 41056.5)^-7.35413))^0.114205

FitProb. = 0.0013, ResVar. = 6.6694

Figure 8.6-23 – Standard curves for VEGF generated from Luminex assays

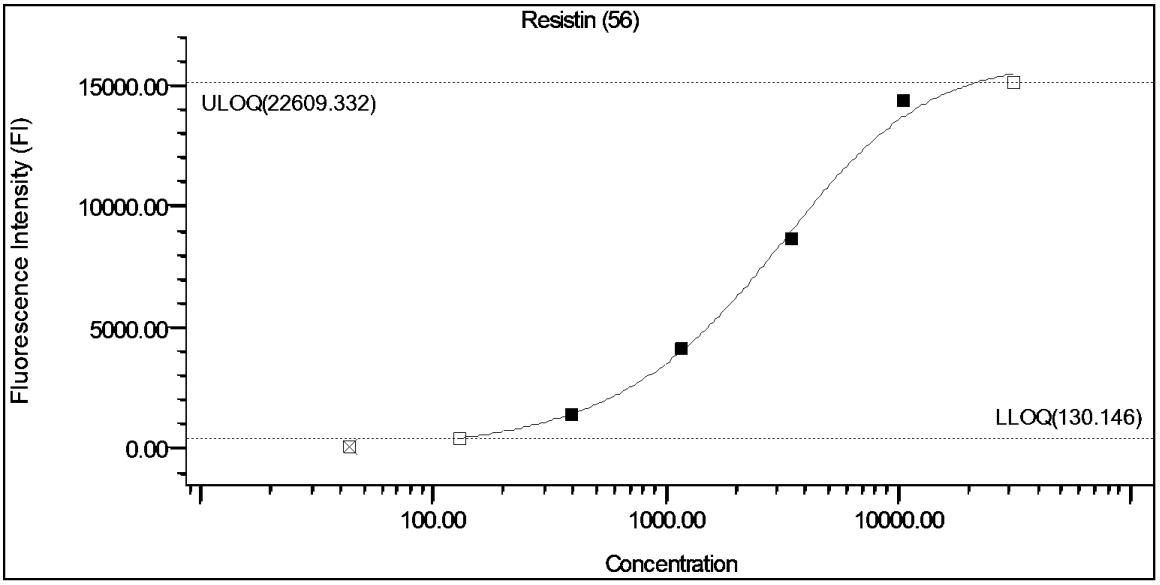
8.6.7 Human obesity premixed ELISA

Resistin and leptin were measured using Human Obesity Premixed Magnetic Luminex Performance Assay Kit (P205396, R&D systems, Bio-techne). Samples were diluted 1:4 with calibrator diluent RD6-46. After preparation of reagents and standards, 50µL of standards and diluted samples was pipetted into each well of the 96T micro-titre plates. Samples were pipetted in singlicate across two duplicate plates. Standards were pipetted in duplicate on both plates. Subsequently, 50µL of diluted microparticle cocktail was added to each well of the micro-titre plates. Plates were covered and incubated for 180minutes at room temperature on a horizontal orbital plate shaker set at 800rpm. Following this, solution was aspirated from the wells and the plates were washed with wash buffer three times using an electronic plate washer with a magnetic plate holder (R&D systems). Next, 50µL of diluted Biotin-Antibody cocktail was added to all wells, the plates were covered with a sealer and incubated for 60 minutes at room temperature on the shaker set at 800rpm. The wash process was then repeated. Following this 50µL of diluted Streptavidin-PE was pipetted to all wells. Plates were again covered with a sealer and incubated for 30minutes at room temperature on the shaker at 800rpm. The wash process was again repeated after this. Finally, the microparticles were resuspended by adding 100µL of wash buffer to each well. Plates were incubated on the shaker set at 800rpm for two minutes. Plates were read using a Bio-Rad analyser. Standard curves generated from the assay are shown in Figure 8.6-24 and Figure 8.6-25.



Regression Type: Logistic - 5PL
Std. Curve: FI = -1.71355 + (18084.3 + 1.71355) / ((1 + (Conc / 10940.6)^-1.31531))^1.23403
FitProb. = 0.0171, ResVar. = 4.0667

Figure 8.6-24 – Standard curves for Leptin generated from Luminex assays



Regression Type: Logistic - 5PL
Std. Curve: FI = -87.4985 + (16032.9 + 87.4985) / ((1 + (Conc / 4538.22)^-1.51116))^0.630189
FitProb. = 0.1252, ResVar. = 2.3506

Figure 8.6-25 – Standard curves generated for Resistin from Luminex assays

8.6.8 Variables included in analysis

Table 8.6-1 shows all the variables which were initially imported for visual inspection of data, and the definitions of these. Biomarkers that did not show differentiation between participants were not included at this stage.

Table 8.6-2 demonstrates the variables that were selected as features within the LASSO and Elastic Net models and subsequent network analysis.

Table 8.6-1 – Variables initially imported for visual inspection.

Variable name	Type	Description
Group	Binary	0=emergency surgery, 1=medical, 2=elective surgery
Age	Continuous	In years
Sex	Binary	0=female, 1=male
Ethnicity	Categorical	0=White British, 1=White Irish, 2=Indian, 3=Arab
Smoking	Ordinal	0=Non-smoker, 1=Ex-smoker, 3=Current smoker
DM	Binary	Diabetes Mellitus; 1=yes, 0=no
HF	Binary	Heart failure; 1=yes, 0=no
IHD	Binary	Ischaemic Heart Disease; 1=yes, 0=no
Stroke	Binary	Previous stroke; 1=yes, 0=no
Cancer	Binary	Active or recently treated; 1=yes, 0=no
Asthma	Binary	Asthma; 1=yes, 0=no
COPD	Binary	Chronic Obstructive Pulmonary Disease; 1=yes, 0=no
Anx_Dep	Binary	Anxiety/depression; 1=yes, 0=no
Cognitive	Binary	Pre-existent cognitive impairment; 1=yes, 0=no
Infection	Categorical	(Medical patients only): Infection type 1=Respiratory, 2=Urinary, 3=Skin, 4=Biliary, 5=COVID-19, 6=Unknown source
Lap_Open	Binary	(Surgical patients only): Operation type 1=Laparoscopic, 2=Open
Digoxin	Binary	Treatment with prior to or during hospitalisation; 1=yes, 0=no
Metformin	Binary	Treatment with prior to or during hospitalisation; 1=yes, 0=no
Statin	Binary	Treatment with prior to or during hospitalisation; 1=yes, 0=no
Steroids	Binary	Treatment with prior to or during hospitalisation; 1=yes, 0=no
ADLs_Baseline	Continuous	Combined score of Katz (scored out of 6) and Lawton (scored out of 8) ADLs - Baseline
ADLs_V3	Continuous	Combined score of Katz (scored out of 6) and Lawton (scored out of 8) ADLs - 7 days
ADLs_V4	Continuous	Combined score of Katz (scored out of 6) and Lawton (scored out of 8) ADLs - 13 weeks
BMI_V1	Continuous	Body Mass Index - Baseline
BMI_V4	Continuous	Body Mass Index - 13 weeks
MNA_V1	Continuous	Mini Nutritional Assessment - Baseline (max score 30)
MNA_V4	Continuous	Mini Nutritional Assessment - 13 weeks (max score 30)

Nutrition_V1	Ordinal	Categorised MNA Baseline; 0=normal, 1=at risk, 2=malnourished *Calculated using recognised cut-offs from MNA*
Nutrition_V4	Ordinal	Categorised MNA 13 weeks; 0=normal, 1=at risk, 2=malnourished *Calculated using recognised cut-offs from MNA*
Steps_count	Continuous	Average steps/day in hospital from Fitbit
Steps_900	Binary	Categorised from above; 0=less than 900, 1=900 or greater steps/day
Delirium	Binary	Delirium during admission; 0=no, 1=yes
LoS	Continuous	Length of stay in whole days
Hospital_Total	Continuous	Total days in acute hospital from baseline assessment to 13 weeks (including readmissions)
Death_IP	Binary	Death during admission; 0=no, 1=yes
TBW_V1	Continuous	Total body water (bioelectrical impedance analysis) - baseline
TBW_V2	Continuous	Total body water (bioelectrical impedance analysis) - within 48 hours of surgery (elective only)
TBW_V3	Continuous	Total body water (bioelectrical impedance analysis) - 7 days post-operative/admission
TBW_V4	Continuous	Total body water (bioelectrical impedance analysis) - 13 weeks post-operative/admission
TBW%_V1	Continuous	TBW as % of total weight (bioelectrical impedance analysis) - baseline
TBW%_V2	Continuous	TBW as % of total weight (bioelectrical impedance analysis) - 48 hours (elective only)
TBW%_V3	Continuous	TBW as % of total weight (bioelectrical impedance analysis) - 7 days
TBW%_V4	Continuous	TBW as % of total weight(bioelectrical impedance analysis) - 13 weeks
ECW_V1	Continuous	Extracellular water (bioelectrical impedance analysis) - baseline
ECW_V2	Continuous	Extracellular water(bioelectrical impedance analysis) - 48 hours (elective only)
ECW_V3	Continuous	Extracellular water (bioelectrical impedance analysis) - 7 days
ECW_V4	Continuous	Extracellular water (bioelectrical impedance analysis) - 13 weeks
ECW%_V1	Continuous	ECW as % of total weight (bioelectrical impedance analysis) - baseline
ECW%_V2	Continuous	ECW as % of total weight (bioelectrical impedance analysis) - 48 hours (elective only)
ECW%_V3	Continuous	ECW as % of total weight (bioelectrical impedance analysis) - 7 days
ECW%_V4	Continuous	ECW as % of total weight (bioelectrical impedance analysis) - 13 weeks

ICW_V1	Continuous	Intracellular water (bioelectrical impedance analysis) - baseline
ICW_V2	Continuous	Intracellular water(bioelectrical impedance analysis) - 48 hours (elective only)
ICW_V3	Continuous	Intracellular water (bioelectrical impedance analysis) - 7 days
ICW_V4	Continuous	Intracellular water (bioelectrical impedance analysis) - 13 weeks
ICW%_V1	Continuous	ICW as % of total weight (bioelectrical impedance analysis) - baseline
ICW%_V2	Continuous	ICW as % of total weight (bioelectrical impedance analysis) - 48 hours (elective only)
ICW%_V3	Continuous	ICW as % of total weight (bioelectrical impedance analysis) - 7 days
ICW%_V4	Continuous	ICW as % of total weight (bioelectrical impedance analysis) - 13 weeks
PROMIS_Baseline	Continuous	Patient reported outcome measures information system physical function Z score - Baseline
PROMIS_V3	Continuous	Patient reported outcome measures information system physical function Z score - 7 days
PROMIS_V4	Continuous	Patient reported outcome measures information system physical function Z score - 13 weeks
BATT_V1	Continuous	Bilateral Anterior Thigh Thickness (ultrasound) - Baseline
BATT_V2	Continuous	Bilateral Anterior Thigh Thickness (ultrasound) - 48 hours (elective only)
BATT_V3	Continuous	Bilateral Anterior Thigh Thickness (ultrasound) - 7 days
BATT_V4	Continuous	Bilateral Anterior Thigh Thickness (ultrasound) - 13 weeks
BATTSCR_V1	Continuous	BATT: Subcutaneous Ratio (ultrasound) - Baseline
BATTSCR_V2	Continuous	BATT: Subcutaneous Ratio (ultrasound) - 48 hours (elective only)
BATTSCR_V3	Continuous	BATT: Subcutaneous Ratio (ultrasound) - 7 days
BATTSCR_V4	Continuous	BATT: Subcutaneous Ratio (ultrasound) - 13 weeks
Echo_V1	Continuous	Rectus femoris echogenicity (ultrasound gray scale) - Baseline
Echo_V2	Continuous	Rectus femoris echogenicity (ultrasound gray scale) - 48 hours (elective only)
Echo_V3	Continuous	Rectus femoris echogenicity (ultrasound gray scale) - 7 days
Echo_V4	Continuous	Rectus femoris echogenicity (ultrasound gray scale) - 13 weeks
SMMSergi_V1	Continuous	Skeletal Muscle Mass (Sergi equation) (Bioelectrical impedance analysis) - Baseline
SMMSergi_V2	Continuous	Skeletal Muscle Mass (Sergi equation) (Bioelectrical impedance analysis) - 48 hours (elective)

SMMSergi_V3	Continuous	Skeletal Muscle Mass (Sergi equation) (Bioelectrical impedance analysis) - 7 days
SMMSergi_V4	Continuous	Skeletal Muscle Mass (Sergi equation) (Bioelectrical impedance analysis) - 13 weeks
SMMJanssen_V1	Continuous	Skeletal Muscle Mass (Janssen equation) (Bioelectrical impedance analysis) - Baseline
SMMJanssen_V2	Continuous	Skeletal Muscle Mass (Janssen equation) (Bioelectrical impedance analysis) - 48 hours (elective)
SMMJanssen_V3	Continuous	Skeletal Muscle Mass (Janssen equation) (Bioelectrical impedance analysis) - 7 days
SMMJanssen_V4	Continuous	Skeletal Muscle Mass (Janssen equation) (Bioelectrical impedance analysis) - 13 weeks
PA_V1	Continuous	Phase angle (Bioelectrical impedance analysis) - Baseline
PA_V2	Continuous	Phase angle (Bioelectrical impedance analysis) - 48 hours (elective)
PA_V3	Continuous	Phase angle (Bioelectrical impedance analysis) - 7 days
PA_V4	Continuous	Phase angle (Bioelectrical impedance analysis) - 13 weeks
HGS_V1	Continuous	Handgrip strength - Baseline
HGS_V2	Continuous	Handgrip strength - 48 hours (elective)
HGS_V3	Continuous	Handgrip strength - 7 days
HGS_V4	Continuous	Handgrip strength - 13 weeks
WS_V1	Continuous	Gait (walking) speed - Baseline
WS_V3	Continuous	Gait (walking) speed - 7 days
WS_V4	Continuous	Gait (walking) speed - 13 weeks
SPPB_V1	Continuous	Short Physical Perfomance Battery - Baseline S*core 0 to 12, derived from continuous variables, of which gait speed is one of, and normally analysed as continuous*
SPPB_V3	Continuous	Short Physical Perfomance Battery - 7 days
SPPB_V4	Continuous	Short Physcial Performance Battery - 13 weeks
V1_CFS	Ordinal	Clinical Frailty Scale - Baseline *Scored 1 (very fit) to 8 (very severely frail)*
V3_CFS	Ordinal	Clinical Frailty Scale - 7 days
V4_CFS	Ordinal	Clinical Frailty Scale - 13 weeks
V1_CFS_Frail	Binary	Frailty defined by CFS - Baseline; 0=no, 1=yes *Frailty defined as CFS greater than or equal to 5*
V3_CFS_Frail	Binary	Frailty defined by CFS - 7 days; 0=no, 1=yes
V4_CFS_Frail	Binary	Frailty defined by CFS - 13 weeks; 0=no, 1=yes
V1_FI	Continuous	Frailty index - Baseline *Derived from 36 separate variables - count of these divided by 36 gives index between 0 and 1*
V3_FI	Continuous	Frailty index - 7 days
V4_FI	Continuous	Frailty index - 13 weeks

V1_FI_Frail	Binary	Frailty defined by FI - Baseline; 0=no, 1=yes *Frailty defined by FI greater than or equal to 0.25*
V3_FI_Frail	Binary	Frailty defined by FI - 7 days; 0=no, 1=yes
V4_FI_Frail	Binary	Frailty defined by FI - 13 weeks; 0=no, 1=yes
V1_Fried_Frail	Binary	Frailty defined by Fried - Baseline; 0=no, 1=yes *Frailty defined as three or more of: low handgrip strength (defined cut-offs), low gait speed (defined cut-offs), weight loss >4.5kg/5% over last year, self-reported exhaustion, low physical activity*
V3_Fried_Frail	Binary	Frailty defined by FI - 7 days; 0=no, 1=yes
V4_Fried_Frail	Binary	Frailty defined by FI - 13 weeks; 0=no, 1=yes
V1_Sarc_Any	Binary	Sarcopenia - Baseline; 0=no, 1=yes *Defined as 1) handgrip strength below recognised cut-off AND 2) BATT below recognised or cut-off OR SMMSergi below recognised cut-off*
V3_Sarc_Any	Binary	Sarcopenia - 7 days; 0=no, 1=yes
V4_Sarc_Any	Binary	Sarcopenia - 13 weeks; 0=no, 1 yes
Hb_V0	Continuous	Haemoglobin - Preoperative (routine clinical bloods)
Hb_V1	Continuous	Haemoglobin - within 48 hours of admission or surgery (routine clinical bloods)
Hb_V3	Continuous	Haemoglobin - 7 days (routine clinical bloods)
Creat_V0	Continuous	Creatinine - Preoperative (routine clinical bloods)
Creat_V1	Continuous	Creatinine - within 48 hours of admission or surgery (routine clinical bloods)
Creat_V3	Continuous	Creatinine - 7 days (routine clinical bloods)
eGFR_V0	Continuous	Glomerular Filtration Rate - Preoperative (routine clinical bloods)
eGFR_V1	Continuous	Glomerular Filtration Rate - within 48 hours of admission or surgery (routine clinical bloods)
eGFR_V3	Continuous	Glomerular Filtration Rate - 7 days (routine clinical bloods)
CRP_V0	Continuous	C-Reactive Protein - preoperative (from hsCRP ELISA - Elective, or routine clinical bloods - emergency surgery
CRP_V1	Continuous	C-reactive Protein - within 48 hours of admission or surgery (routine clinical bloods)
CRP_V3	Continuous	C-reactive Protein - 7 days (routine clinical bloods)
Alb_V0	Continuous	Albumin - Preoperative (routine clinical bloods)
Alb_V1	Continuous	Albumin - within 48 hours of admission or surgery (routine clinical bloods)
Alb_V3	Continuous	Albumin - 7 days (routine clinical bloods)
WCC_V0	Continuous	White cell count - Preoperative (routine clinical bloods)
WCC_V1	Continuous	White cell count - Within 48 hours of admission or surgery (routine clinical bloods)
WCC_V3	Continuous	White cell count - 7 days (routine clinical bloods)

Neu_V0	Continuous	Neutrophil count - Preoperative (routine clinical bloods)
Neu_V1	Continuous	Neutrophil count - Within 48 hours of admission or surgery (routine clinical bloods)
Neu_V3	Continuous	Neutrophil count - 7 days (routine clinical bloods)
Lym_V0	Continuous	Lymphocyte count - Preoperative (routine clinical bloods)
Lym_V1	Continuous	Lymphocyte count - Within 48 hours of admission or surgery (routine clinical bloods)
Lym_V3	Continuous	Lymphocyte count - 7 days (routine clinical bloods)
Myostatin_V0	Continuous	Myostatin - Preoperative (ELISA)
Myostatin_V1	Continuous	Myostatin - Within 48 hours of admission or surgery (ELISA)
Cortisol_V0	Continuous	Cortisol - Preoperative (ELISA)
Cortisol_V1	Continuous	Cortisol - Within 48 hours of admission or surgery (ELISA)
DHEAS_V0	Continuous	Dehydroepiandrosterone sulfate - Preoperative (ELISA)
DHEAS_V1	Continuous	Dehydroepiandrosterone sulfate - Within 48 hours of admission or surgery (ELISA)
IGF-1_V0	Continuous	Insulin-like growth factor 1 - Preoperative (ELISA)
IGF-1_V1	Continuous	Insulin-like growth factor 1 - Within 48 hours of admission or surgery (ELISA)
GH_V0	Continuous	Growth Hormone - Preoperative (ELISA)
GH_V1	Continuous	Growth Hormone - Within 48 hours of admission or surgery (ELISA)
VitD_V0	Continuous	25-OH Vitamin D - Preoperative (ELISA)
VitD_V1	Continuous	25-OH Vitamin D - Within 48 hours of admission or surgery (ELISA)
CCL2_V0	Continuous	Luminex
CCL2_V1	Continuous	Luminex
CXCL10_V0	Continuous	Luminex
CXCL10_V1	Continuous	Luminex
IL-1a_V0	Continuous	Luminex
IL-1a_V1	Continuous	Luminex
IL-6_V0	Continuous	Luminex
IL-6_V1	Continuous	Luminex
IL-10_V0	Continuous	Luminex
IL-10_V1	Continuous	Luminex
VEGF_V0	Continuous	Luminex
VEGF_V1	Continuous	Luminex
IL-1b_V0	Continuous	Luminex
IL-1b_V1	Continuous	Luminex
IL-7_V0	Continuous	Luminex
IL-7_V1	Continuous	Luminex
IL-15_V0	Continuous	Luminex

IL-15_V1	Continuous	Luminex
CXCL1_V0	Continuous	Luminex
CXCL1_V1	Continuous	Luminex
IL-8_V0	Continuous	Luminex
IL-8_V1	Continuous	Luminex
TNFa_V0	Continuous	Luminex
TNFa_V1	Continuous	Luminex
Leptin_V0	Continuous	Luminex
Leptin_V1	Continuous	Luminex
Resistin_V0	Continuous	Luminex
Resistin_V1	Continuous	Luminex

Table 8.6-2 – Features included in analysis.

Features that were included if fewer than 30% missing variables were present are shown in bold. Features that were specifically considered in the second analysis focusing on participants where these variables were present are highlighted in light grey.

Group	0=emergency surgery, 1=medical, 2=elective surgery
Age	In years
Sex	0=female, 1=male
Ethnicity	0=White British, 1=White Irish, 2=Indian, 3=Arab
Smoking	0=Non-smoker, 1=Ex-smoker, 3=Current smoker
DM	Diabetes Mellitus; 1=yes, 0=no
HF	Heart failure; 1=yes, 0=no
IHD	Ischaemic Heart Disease; 1=yes, 0=no
Stroke	Previous stroke; 1=yes, 0=no
Cancer	Active or recently treated; 1=yes, 0=no
Asthma	Asthma; 1=yes, 0=no
COPD	Chronic Obstructive Pulmonary Disease; 1=yes, 0=no
Anx_Dep	Anxiety/depression; 1=yes, 0=no
Cognitive	Pre-existent cognitive impairment; 1=yes, 0=no
Infection	(Medical patients only): Infection type 1=Respiratory, 2=Urinary, 3=Skin, 4=Biliary, 5=COVID-19, 6=Unknown source
Lap_Open	(Surgical patients only): Operation type 1=Laparoscopic, 2=Open
Digoxin	Treatment with prior to or during hospitalisation; 1=yes, 0=no
Metformin	Treatment with prior to or during hospitalisation; 1=yes, 0=no
Statin	Treatment with prior to or during hospitalisation; 1=yes, 0=no
Steroids	Treatment with prior to or during hospitalisation; 1=yes, 0=no
BMI_V1	Body Mass Index - Baseline
BMI_V4	Body Mass Index - 13 weeks
MNA_V1	Mini Nutritional Assessment - Baseline (max score 30)
MNA_V4	Mini Nutritional Assessment - 13 weeks (max score 30)
Nutrition_V1	Categorised MNA Baseline; 0=normal, 1=at risk, 2=malnourished *Calculated using recognised cut-offs from MNA*
Nutrition_V4	Categorised MNA 13 weeks; 0=normal, 1=at risk, 2=malnourished *Calculated using recognised cut-offs from MNA*
Steps_count	Average steps/day in hospital from Fitbit
Steps_900	Categorised from above; 0=less than 900, 1=900 or greater steps/day
Delirium	Delirium during admission; 0=no, 1=yes
LoS	Length of stay in whole days
Hospital_Total	Total days in acute hospital from baseline assessment to 13 weeks (including readmissions)
Hb_V0	Haemoglobin - Preoperative (routine clinical bloods)
Hb_V1	Haemoglobin - within 48 hours of admission or surgery (routine clinical bloods)
Hb_V3	Haemoglobin - 7 days (routine clinical bloods)

Creat_V0	Creatinine - Preoperative (routine clinical bloods)
Creat_V1	Creatinine - within 48 hours of admission or surgery (routine clinical bloods)
Creat_V3	Creatinine - 7 days (routine clinical bloods)
eGFR_V0	Glomerular Filtration Rate - Preoperative (routine clinical bloods)
eGFR_V1	Glomerular Filtration Rate - within 48 hours of admission or surgery (routine clinical bloods)
eGFR_V3	Glomerular Filtration Rate - 7 days (routine clinical bloods)
CRP_V0	C-Reactive Protein - preoperative (from hsCRP ELISA - Elective, or routine clinical bloods - emergency surgery
CRP_V1	C-reactive Protein - within 48 hours of admission or surgery (routine clinical bloods)
CRP_V3	C-reactive Protein - 7 days (routine clinical bloods)
Alb_V0	Albumin - Preoperative (routine clinical bloods)
Alb_V1	Albumin - within 48 hours of admission or surgery (routine clinical bloods)
Alb_V3	Albumin - 7 days (routine clinical bloods)
WCC_V0	White cell count - Preoperative (routine clinical bloods)
WCC_V1	White cell count - Within 48 hours of admission or surgery (routine clinical bloods)
WCC_V3	White cell count - 7 days (routine clinical bloods)
Neu_V0	Neutrophil count - Preoperative (routine clinical bloods)
Neu_V1	Neutrophil count - Within 48 hours of admission or surgery (routine clinical bloods)
Neu_V3	Neutrophil count - 7 days (routine clinical bloods)
Lym_V0	Lymphocyte count - Preoperative (routine clinical bloods)
Lym_V1	Lymphocyte count - Within 48 hours of admission or surgery (routine clinical bloods)
Lym_V3	Lymphocyte count - 7 days (routine clinical bloods)
Myostatin_V0	Myostatin - Preoperative (ELISA)
Myostatin_V1	Myostatin - Within 48 hours of admission or surgery (ELISA)
Cortisol_V0	Cortisol - Preoperative (ELISA)
Cortisol_V1	Cortisol - Within 48 hours of admission or surgery (ELISA)
DHEAS_V0	Dehydroepiandrosterone sulfate - Preoperative (ELISA)
DHEAS_V1	Dehydroepiandrosterone sulfate - Within 48 hours of admission or surgery (ELISA)
IGF-1_V0	Insulin-like growth factor 1 - Preoperative (ELISA)
IGF-1_V1	Insulin-like growth factor 1 - Within 48 hours of admission or surgery (ELISA)
GH_V0	Growth Hormone - Preoperative (ELISA)
GH_V1	Growth Hormone - Within 48 hours of admission or surgery (ELISA)
VitD_V0	25-OH Vitamin D - Preoperative (ELISA)
VitD_V1	25-OH Vitamin D - Within 48 hours of admission or surgery (ELISA)
CCL2_V0	Luminex
CCL2_V1	Luminex

CXCL10_V0	Luminex
CXCL10_V1	Luminex
IL-1a_V0	Luminex
IL-1a_V1	Luminex
IL-6_V0	Luminex
IL-6_V1	Luminex
IL-10_V0	Luminex
IL-10_V1	Luminex
VEGF_V0	Luminex
VEGF_V1	Luminex
IL-1b_V0	Luminex
IL-1b_V1	Luminex
IL-7_V0	Luminex
IL-7_V1	Luminex
IL-15_V0	Luminex
IL-15_V1	Luminex
CXCL1_V0	Luminex
CXCL1_V1	Luminex
IL-8_V0	Luminex
IL-8_V1	Luminex
TNFa_V0	Luminex
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Resistin_V0	Luminex
Resistin_V1	Luminex

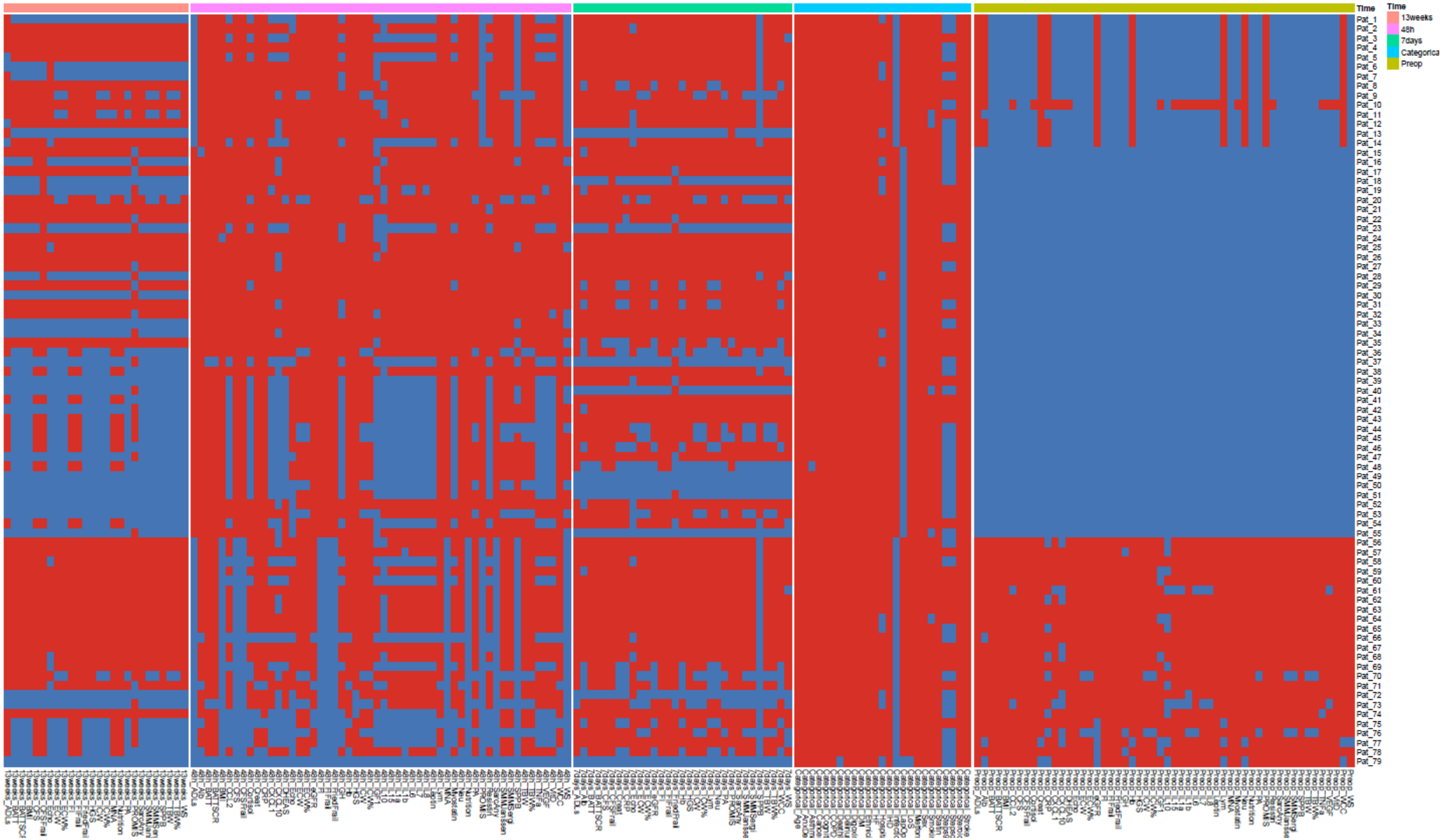

Figure 8.6-26 – – Heatmap showing missing variables.

Missing variables are shown in blue, variables that were available are shown in red.

8.6.9 Supplementary results

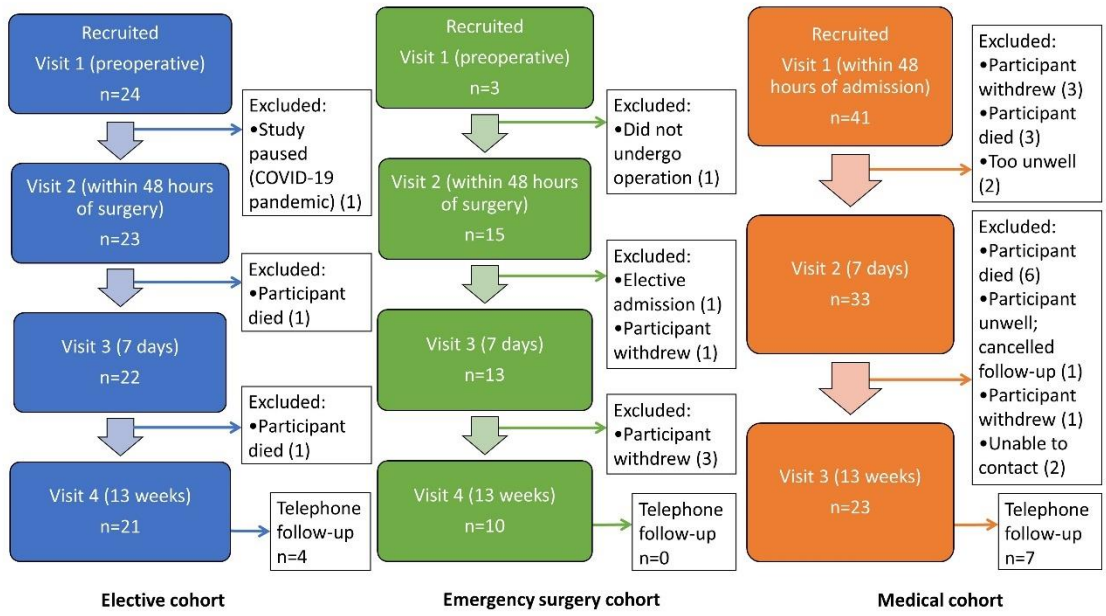

Figure 8.6-27 – Recruitment flowchart for participants separated by group

Table S8.6-3 – Beta coefficients derived from LASSO and Elastic Net models for outcomes at timepoints, without specific focus on participants with additional systemic biomarkers available. Results are adjusted for baseline sarcopenia status. Square brackets denote confidence intervals for coefficients. Curved brackets denote the number of models that the association was encountered within, and the number of models that the association was tested within. The timing of the individual variables and outcomes tested are denoted in the first column and row respectively. Variables without timing specified in the first column are constants. The separate timing (second) column refers to the timing of other variables that the associated was tested against. Non-significant associations are not shown.

	Timing	BATT (7 days)	SMMSergi (7 days)	Echogenicity (7 days)	Sarcopenia (7 days)	Sarcopenia (13 weeks)
Age	Preop			0.11 [0.06, 0.16] (18/36)		
	13 weeks				0.14 [0.02, 0.27] (9/22)	0.12 [0.02, 0.21] (5/23)
Anxiety/ Depression	Preop		0.11 [0.04, 0.17] (9/36)		0.44 [0.17, 0.72] (11/18)	
	7 days		0.22 [0.17, 0.27] (40/79)			

Asthma	Preop			0.62 [0.44, 0.81] (24/36)		
	48 hours				0.77 [0.61, 0.92] (64/70)	
	7 days				0.75 [0.62, 0.88] (52/70)	
BATT (48 hours)	48 hours				-0.17 [-0.23, -0.1] (52/70)	
BATT (7 days)	7 days				-0.23 [-0.26, -0.2] (46/70)	
BATT (13 weeks)	13 weeks					-0.23 [-0.35, -0.1] (12/23)
Cancer	Preop			-0.22 [-0.29, -0.16] (25/36)		-0.41 [-0.53, -0.3] (11/20)
COPD	Preop			0.61 [0.51, 0.72] (25/36)	1.05 [0.86, 1.24] (16/18)	-0.47 [-0.7, -0.25] (12/20)
	48 hours	-0.32 [-0.34, -0.29] (77/79)	-0.29 [-0.31, -0.26] (68/79)		0.67 [0.59, 0.75] (66/70)	
	7 days	-0.36 [-0.38, -0.33] (78/79)	-0.31 [-0.34, -0.28] (65/79)		0.89 [0.71, 1.06] (63/70)	
	13 weeks	-0.67 [-0.7, -0.64] (78/79)	-0.56 [-0.6, -0.53] (79/79)		1.37 [1.09, 1.66] (22/22)	
Creatinine (Preop)	Preop	0.17 [0.15, 0.2] (36/36)	0.25 [0.19, 0.31] (22/36)			
CRP (48 hours)	48 hours	-0.04 [-0.05, -0.03] (34/79)				
Delirium	13 weeks				-1.15 [-1.95, -0.35] (8/22)	
Digoxin	13 weeks				-1.42 [-2.02, -0.82] (20/22)	
Diabetes Mellitus	48 hours	0.39 [0.36, 0.43] (79/79)	0.13 [0.1, 0.16] (49/79)			
	7 days	0.41 [0.38, 0.43] (79/79)	0.14 [0.12, 0.17] (53/79)			
	13 weeks	0.42 [0.39, 0.45] (77/79)	0.16 [0.13, 0.2] (65/79)		-0.72 [-1.02, -0.42] (13/22)	-0.64 [-0.78, -0.5] (13/23)
eGFR	48 hours	-0.05	-0.12			

(48 hours)		[-0.06, -0.04] (53/79)	[-0.14, -0.11] (64/79)			
eGFR (7days)	7 days	-0.16 [-0.17, -0.15] (79/79)	-0.21 [-0.23, -0.2] (74/79)			
eGFR (Preop)	Preop			0.39 [0.27, 0.5] (24/36)		
White British ethnicity	13weeks					-0.24 [-0.32, -0.15] (15/23)
Hb (7 days)	7 days	-0.05 [-0.07, -0.04] (35/79)				
Handgrip strength (48 hours)	48 hours	0.22 [0.21, 0.23] (79/79)	0.17 [0.16, 0.18] (75/79)		-0.91 [-1.01, -0.81] (70/70)	
Handgrip strength (7 days)	7 days	0.28 [0.27, 0.29] (79/79)	0.26 [0.25, 0.27] (75/79)		-1.25 [-1.42, -1.08] (70/70)	
Handgrip strength (13 weeks)	13 weeks				-0.63 [-0.86, -0.39] (21/22)	-0.57 [-0.74, -0.41] (17/23)
Ischaemic Heart Disease	48 hours	0.16 [0.14, 0.19] (62/79)				
	7 days	0.21 [0.18, 0.24] (62/79)			0.7 [0.55, 0.85] (40/70)	
	13 weeks	0.28 [0.25, 0.3] (73/79)				
Length of stay	Preop			-0.16 [-0.24, -0.08] (19/36)		
Lymphocytes (Preop)	Preop			-0.13 [-0.16, -0.09] (22/36)		
Lymphocytes (7 days)	7 days	-0.06 [-0.07, -0.04] (47/79)				
Metformin	Preop			1.11 [0.8, 1.42] (25/36)		
	7 days	0.25 [0.18, 0.31] (36/79)				
	13 weeks	0.2 [0.14, 0.26] (43/79)				
Nutrition (13weeks): At Risk (vs malnourished)	13 weeks				0.46 [0.32, 0.6] (21/22)	
Phase Angle	13 weeks				-0.19	-0.06

(13 weeks)					[-0.25, -0.14] (20/22)	[-0.1, -0.02] (7/23)
Phase Angle (48 hours)	48 hours	0.06 [0.05, 0.06] (63/79)			-0.26 [-0.47, -0.06] (62/70)	
Phase Angle (7 days)	7 days	0.09 [0.08, 0.1] (72/79)			-0.58 [-0.73, -0.43] (47/70)	
PROMIS Physical Function (Preop)	Preop				-0.38 [-0.52, -0.25] (16/18)	
Sex (male)	Preop	0.1 [0.07, 0.13] (29/36)	0.08 [0.06, 0.11] (18/36)		0.2 [0.08, 0.33] (11/18)	-0.22 [-0.37, -0.07] (8/20)
	48 hours		0.15 [0.14, 0.17] (73/79)		0.27 [0.21, 0.34] (64/70)	
	7 days		0.12 [0.11, 0.14] (70/79)		0.56 [0.47, 0.65] (61/70)	
	13 weeks	0.15 [0.14, 0.17] (77/79)	0.33 [0.3, 0.35] (79/79)		0.84 [0.42, 1.26] (8/22)	0.45 [0.17, 0.74] (11/23)
SMMSergi (13 weeks)	13 weeks				-0.23 [-0.27, -0.18] (14/22)	
Ex-smoker (vs current smoker)	Preop					-0.22 [-0.34, -0.09] (5/20)
Non-smoker (vs current smoker)	Preop					-0.35 [-0.57, -0.14] (8/20)
	7 days	-0.15 [-0.19, -0.11] (39/79)				
	13 weeks	-0.19 [-0.22, -0.17] (59/79)				
Steroids	Preop			-0.25 [-0.36, -0.14] (21/36)	0.43 [0.26, 0.59] (10/18)	
	48 hours				1.11 [0.98, 1.24] (64/70)	
	7 days				0.75 [0.61, 0.89] (58/70)	
	13 weeks				1.18 [0.84, 1.52] (19/22)	
Stroke	7 days	0.23 [0.16, 0.3] (46/79)				
	13 weeks	0.21				

		[0.12, 0.3] (33/79)				
White Cell Count (Preop)	Preop			0.35 [0.13, 0.57] (24/36)		
Walking Speed (13 weeks)	13 weeks				-0.2 [-0.37, -0.04] (6/22)	

Table S8.6-4 – Beta coefficients derived from LASSO and Elastic Net models for outcomes at timepoints, with specific focus on participants with additional systemic biomarkers available. Results are adjusted for baseline sarcopenia status. Square brackets denote confidence intervals for coefficients. Curved brackets denote the number of models that the association was encountered within, and the number of models that the association was tested within. The timing of the individual variables and outcomes tested are denoted in the first column and row respectively. Variables without timing specified in the first column are constants. The separate timing (second) column refers to the timing of other variables that the associated was tested against. Non-significant associations are not shown.

	Timing	BATT (7 days)	BATT (13 weeks)	SMMSergi (7 days)	Echo (7 days)	Sarc (7 days)	Sarc (13 weeks)
ADLs (Preop)	Preop	0.16 [0.1, 0.22] (8/23)					
Anxiety/ Depression	Preop				-0.6 [-0.83, -0.36] (8/23)		
Asthma	Preop						-0.9 [-1.63, -0.17] (3/11)
BATT (48 hours)	48 hours					-0.12 [-0.18, -0.06] (21/35)	
BMI (Preop)	Preop		0.21 [0.14, 0.27] (9/23)	0.13 [0.11, 0.15] (17/23)			
Cancer	Preop						-0.24 [-0.32, -0.17] (6/11)
CCL2 (Preop)	Preop			-0.17 [-0.26, -0.08] (6/23)			
COPD	Preop				0.41 [0.29, 0.54] (8/23)		
	48 hours	-0.43		-0.37	0.22	0.6	

		[-0.46, -0.41] (50/50)		[-0.39, -0.34] (50/50)	[0.18, 0.25] (44/50)	[0.48, 0.71] (34/35)	
Cortisol (Preop)	Preop	-0.18 [-0.24, -0.11] (11/23)		-0.08 [-0.12, -0.04] (9/23)			
Creatinine (Preop)	Preop	0.24 [0.12, 0.37] (15/23)	0.21 [0.01, 0.42] (5/23)	0.35 [0.3, 0.39] (19/23)	-0.16 [-0.23, -0.08] (9/23)		
Digoxin	48 hours	-0.31 [-0.35, -0.28] (42/50)					
Diabetes Mellitus	Preop		0.3 [0.13, 0.48] (9/23)	0.15 [0.02, 0.27] (7/23)			
	48 hours	0.32 [0.28, 0.36] (49/50)					
eGFR (Preop)	Preop				0.15 [0.1, 0.2] (9/23)		
eGFR (48 hours)	48 hours			-0.08 [-0.1, -0.07] (46/50)			
GH (Preop)	Preop		0.27 [0.18, 0.35] (5/23)				
HGS (Preop)	Preop			0.1 [0.06, 0.13] (15/23)			
Handgrip strength (48 hours)	48 hours	0.33 [0.32, 0.35] (50/50)		0.2 [0.19, 0.21] (50/50)	-0.1 [-0.11, -0.09] (48/50)	-1.03 [-1.15, -0.92] (35/35)	
Ischaemic Heart Disease	48 hours	0.19 [0.16, 0.22] (46/50)					
IL15 (Preop)	Preop		0.13 [0.02, 0.24] (5/23)				
IL15 (48 hours)	48 hours			-0.07 [-0.08, -0.06] (47/50)			
IL1a	48 hours	-0.08					

(48 hours)		[-0.1, -0.07] (45/50)					
IL1b (48 hours)	48 hours					0.24 [0.06, 0.42] (10/35)	
IL6 (Preop)	Preop			0.13 [0.03, 0.22] (8/23)			
IL7 (Preop)	Preop				0.17 [0.1, 0.24] (9/23)		
Leptin (48 hours)	48 hours	0.34 [0.33, 0.36] (50/50)		0.35 [0.34, 0.36] (50/50)	-0.33 [-0.34, -0.32] (50/50)		
Length of stay	48 hours					0.13 [0.05, 0.21] (15/35)	
Metformin	Preop				0.42 [0.17, 0.67] (10/23)		
Myostatin (48 hours)	48 hours	0.07 [0.06, 0.08] (36/50)					
Phase Angle (48 hours)	48 hours	0.05 [0.04, 0.06] (36/50)				-0.19 [-0.27, -0.12] (27/35)	
Resistin (48 hours)	48 hours	0.05 [0.04, 0.05] (36/50)				-0.12 [-0.23, -0.01] (7/35)	
Sex (male)	48 hours	0.07 [0.06, 0.08] (45/50)		0.23 [0.21, 0.25] (50/50)	-0.07 [-0.08, -0.06] (44/50)		
Statin	Preop		0.07 [0.02, 0.12] (6/23)				
	48 hours				-0.1 [-0.12, -0.08] (39/50)		
Steroids	48 hours					0.78 [0.55, 1.01] (23/35)	
TNFa	48 hours	0.02			-0.02		

(48 hours)		[0.01, 0.03] (19/50)			[-0.03, -0.01] (6/50)		
TNFa (Preop)	Preop	0.17 [0.1, 0.23] (8/23)		0.15 [0.11, 0.19] (12/23)	-0.34 [-0.47, -0.21] (16/23)		
White Cell Count (48 hours)	48 hours						

Table S8.6-5 – Beta coefficients derived from LASSO and Elastic Net models for change in outcomes from baseline, without specific focus on participants with additional systemic biomarkers available. Square brackets denote confidence intervals for coefficients. Curved brackets denote the number of models that the association was encountered within, and the number of models that the association was tested within. The timing of the individual variables and outcomes tested are denoted in the first column and row respectively. Variables without timing specified in the first column are constants. The separate timing (second) column refers to the timing of other variables that the associated was tested against. Non-significant associations are not shown.

	Timing	ΔBATT (7 days)	ΔBATT (13 weeks)	ΔSMMSergi (7 days)	ΔEchogenicity (7 days)
Age	Preop	0.18 [0.13, 0.24] (7/36)	0.19 [0.16, 0.22] (27/36)		
	7 days	0.11 [0.09, 0.12] (68/79)			
Anxiety/ Depression	48 hours			-0.23 [-0.28, -0.18] (14/79)	
BATT (48 hours)	48 hours	-0.11 [-0.15, -0.07] (24/79)			
BATT (7 days)	7 days	0.4 [0.38, 0.42] (76/79)			
Cancer	Preop	0.17 [0.04, 0.29] (5/36)			-0.61 [-1.02, -0.21] (5/36)
	48 hours			0.19 [0.14, 0.24] (13/79)	
	7 days	0.14 [0.12, 0.16] (64/79)			
COPD	7 days	0.23 [0.21, 0.25] (71/79)			
Creatinine (7 days)	7 days			0.41 [0.37, 0.44] (11/79)	

Creatinine (Preop)	Preop				-0.14 [-0.23, -0.05] (6/36)
CRP (48 hours)	48 hours			0.13 [0.11, 0.15] (16/79)	
Delirium	Preop	-0.33 [-0.55, -0.11] (7/36)	-0.84 [-0.93, -0.75] (25/36)		
	7 days	-0.47 [-0.5, -0.44] (72/79)			
Diabetes Mellitus	48 hours			0.48 [0.38, 0.57] (14/79)	
Echo (48 hours)	48 hours			-0.16 [-0.2, -0.12] (21/79)	
eGFR (Preop)	Preop	-0.11 [-0.16, -0.05] (7/36)			0.24 [0.1, 0.38] (8/36)
eGFR (7 days)	7 days	-0.14 [-0.16, -0.13] (68/79)			
Hb (Preop)	Preop	-0.14 [-0.22, -0.06] (8/36)	-0.06 [-0.08, -0.04] (22/36)		
Hb (7days)	7 days	-0.04 [-0.05, -0.04] (55/79)		-0.03 [-0.05, 0] (5/79)	
Handgrip strength (48 hours)	48 hours			-0.06 [-0.08, -0.03] (13/79)	
Handgrip strength (7 days)	7 days			-0.05 [-0.08, -0.02] (7/79)	
Ischaemic Heart Disease	Preop		0.2 [0.14, 0.25] (25/36)		
	48 hours			-0.38 [-0.47, -0.29] (17/79)	
Length of stay	48 hours			-0.15 [-0.18, -0.12] (16/79)	
Metformin	48 hours			-0.54 [-0.67, -0.41] (15/79)	
Neutrophils (7 days)	7 days	0.12 [0.11, 0.14] (52/79)			
Phase Angle (48 hours)	48 hours			-0.2 [-0.24, -0.16] (23/79)	
PROMIS	7 days	0.05			

(7 days)		[0.04, 0.06] (54/79)			
SMMSergi (7 days)	7 days	-0.29 [-0.31, -0.27] (71/79)			
Non-smoker (vs current smoker)	7 days	-0.15 [-0.17, -0.13] (65/79)			
	13 weeks	-0.15 [-0.22, -0.08] (4/79)			
Ex-smoker (vs current smoker)	7 days	0.06 [0.04, 0.07] (47/79)			
Statin	Preop		0.48 [0.44, 0.51] (27/36)		
Steroids	Preop		-0.19 [-0.24, -0.15] (19/36)		
Stroke	7 days	0.29 [0.26, 0.32] (58/79)			
White Cell Count (7 days)	7 days	0.14 [0.12, 0.16] (50/79)			

Table 8.6-6 – Beta coefficients derived from LASSO and Elastic Net models for change in outcomes from baseline, with specific focus on participants with additional systemic biomarkers available. Square brackets denote confidence intervals for coefficients. Curved brackets denote the number of models that the association was encountered within, and the number of models that the association was tested within. The timing of the individual variables and outcomes tested are denoted in the first column and row respectively. Variables without timing specified in the first column are constants. The separate timing (second) column refers to the timing of other variables that the associated was tested against. Non-significant associations are not shown.

	Timing	ΔBATT (7 days)	ΔBATT (13 weeks)	ΔSMMSergi (7 days)	ΔEchogenicity (7 days)
Anxiety/ Depression	Preop				-0.52 [-1.03, -0.01] (3/23)
Asthma	Preop	0.25 [0.07, 0.43] (3/23)			
COPD	Preop				0.35 [0.12, 0.58] (4/23)
Creatinine (48 hours)	48 hours			0.39 [0.31, 0.46] (6/50)	
Delirium	48 hours				0.13 [0.06, 0.2]

					(8/50)
Diabetes Mellitus	48 hours				-0.12 [-0.2, -0.03] (7/50)
eGFR (Preop)	Preop	-0.28 [-0.42, -0.14] (6/23)			
IL8 (48 hours)	48 hours				0.09 [0.07, 0.11] (11/50)
Leptin (48 hours)	48 hours				-0.11 [-0.17, -0.05] (10/50)
Leptin (Preop)	Preop			0.19 [0.04, 0.34] (5/23)	
Lymphocytes (48 hours)	48 hours				-0.12 [-0.16, -0.08] (11/50)
Metformin	Preop				0.21 [0.13, 0.28] (3/23)
Phase Angle (Preop)	Preop			-0.23 [-0.34, -0.13] (7/23)	
Sex	Preop		-0.21 [-0.25, -0.16] (3/23)		
SMMSergi (48 hours)	48 hours				-0.18 [-0.24, -0.13] (9/50)
Steroids	48 hours				-0.25 [-0.34, -0.16] (9/50)
TNFa (48 hours)	48 hours			0.1 [0.07, 0.14] (7/50)	-0.15 [-0.19, -0.11] (12/50)
TNFa (Preop)	Preop			0.27 [0.12, 0.43] (12/23)	-0.27 [-0.4, -0.14] (5/23)
White Cell Count (48 hours)	48 hours				-0.09 [-0.11, -0.06] (6/50)

8.7 Chapter 6.1 – Supplementary information

8.7.1 Appendix 1 – Full search strategy

	Search terms
MEDLINE	1. randomized controlled trial.pt OR controlled clinical trial.pt OR randomized.ti,ab OR randomised.ti,ab OR placebo.ti,ab OR drug therapy.hw OR randomly.ti,ab OR trial.ti,ab OR groups.ti,ab 2. Humans.sh 3. 1 AND 2 4. "Aged".sh OR "Aged, 80 and over".sh OR "Frail Elderly".sh 5. elder*.ti,ab OR septuagenarian*.ti,ab OR octogenarian*.ti,ab OR nonagenarian*.ti,ab OR centenarian*.ti,ab OR older.ti,ab OR geriatric*.ti,ab 6. 4 OR 5 7. "Hospitalization".sh OR "Patient Admission".sh 8. hospital*.ti,ab OR admission*.ti,ab OR inpatient*.ti,ab OR admitted.ti,ab 9. 7 OR 8 10. "Muscular Atrophy".sh 11. (musc* ADJ2 mass).ti,ab OR (musc* ADJ2 size).ti,ab OR (musc* ADJ2 atroph*).ti,ab OR (musc* ADJ2 wast*).ti,ab OR (musc* ADJ2 loss*).ti,ab 12. 10 OR 11 13. "Muscle Weakness".sh 14. (musc* ADJ2 weak*).ti,ab OR (musc* ADJ2 strength).ti,ab OR (musc ADJ2 strong*).ti,ab 15. 13 OR 14 16. “Mobility limitation”.sh 17. (speed* ADJ2 gait).ti,ab OR (walk* ADJ2 speed*).ti,ab OR (physical ADJ performance).ti,ab OR ambulat*.ti,ab or mobil*.ti,ab 18. 16 OR 17 19. 3 AND 6 AND 9 20. 12 OR 15 OR 18

	21. 19 AND 20
EMBASE	1. (random* OR factorial* OR crossover* OR cross over* OR cross-over* OR placebo* OR doubl* blind* OR singl* blind* OR assign* OR allocat* OR volunteer*).af. 2. exp crossover-procedure/ or exp double-blind procedure/ or exp randomized controlled trial/ or exp single-blind procedure/ 3. 1 OR 2 4. (exp geriatric patient/ or exp aged/).ti,ab 5. (elder* OR septuagenarian* OR octogenarian* OR nonagenarian* OR centenarian* OR older OR geriatric*).ti,ab 6. 4 OR 5 7. exp hospitalization/ or exp hospital patient/ or exp hospital admission 8. (hospital* OR admission* OR inpatient* OR admitted).ti,ab 9. 7 OR 8 10. exp muscle atrophy/ or exp muscle mass/ 11. ((musc* ADJ2 mass) OR (musc* ADJ2 size) OR (musc* ADJ2 atroph*) OR (musc* ADJ2 wast*) OR (musc* ADJ2 loss*)).ti,ab 12. 10 OR 11 13. exp muscle weakness/ or exp muscle strength/ 14. ((musc* ADJ2 weak*) OR (musc* ADJ2 strength) OR (musc ADJ2 strong*)).ti,ab 15. 13 OR 14 16. exp physical performance/ or exp walking speed/ 17. ((speed* ADJ2 gait) OR (walk* ADJ2 speed*) OR (physical ADJ performance) OR (ambulat* OR mobil*)).ti,ab 18. 16 OR 17 19. 3 AND 6 AND 9 20. 12 AND 15 AND 18 21. 19 AND 20
CINAHL	1. (MH “Randomized controlled trials”) 2. RCT OR randomised OR randomized OR random OR placebo OR trial OR crossover OR masked OR blind

	3. 1 OR 2 4. (MH “Aged”) OR (MH “Aged, 80 and Over) OR (MH Aged, Hospitalized) OR (MH Frail Elderly) 5. elder* OR septuagenarian* OR octogenarian* OR nonagenarian* OR centenarian* OR older OR geriatric* 6. 4 or 5 7. (MH “Hospitalization”) OR (MH “Patient Admission”) 8. hospital* OR admission* OR inpatient* OR admitted 9. 7 OR 8 10. (MH “Muscular Atrophy”) 11. (musc* N2 mass) OR (musc* N2 size) OR (musc* N2 atroph*) OR (musc* N2 wast*) OR (musc* N2 loss*) 12. 10 OR 11 13. (MH “Muscle weakness”) OR (MH “Muscle strength”) OR (MH “Grip strength”) 14. (musc* N2 weak*) OR (musc* N2 strength) OR (musc N2 strong*) 15. 13 OR 14 16. (MH “Physical Performance) 17. (speed* N2 gait) OR (walk* N2 speed*) OR (physical performance) OR ambulat* OR mobil* 18. 16 OR 17 19. 3 AND 6 AND 9 20. 12 OR 15 OR 18 21. 19 AND 20
Cochrane Library (CENTRAL)	1. [mh "Aged"] OR [mh "Aged, 80 and over"] OR [mh "Frail Elderly"] 2. elder* OR septuagenarian* OR octogenarian* OR nonagenarian*OR centenarian* OR older OR geriatric* 3. 1 OR 2 4. [mh "Hospitalization"] OR [mh "Patient Admission"] 5. hospital* OR admission* OR inpatient* OR admitted 6. 4 OR 5 7. [mh "Muscular Atrophy"]

	8. musc* NEAR/2 mass OR musc* ADJ2 size OR musc* NEAR/2 atroph* OR musc* NEAR/2 wast* OR musc* NEAR/2 loss* 9. 7 OR 8 10. [mh "Muscle Weakness"] 11. musc* NEAR/2 weak* OR musc* NEAR/2 strength OR musc NEAR/2 strong* 12. 10 OR 11 13. [mh "Mobility limitation"] 14. speed* NEAR/2 gait OR walk* NEAR/2 speed* OR physical performance OR ambulat* or mobil* 15. 13 OR 14 16. 3 AND 6 17. 9 OR 12 OR 15 18. 16 AND 17

8.7.2 Appendix 2 – Full study characteristics and results

Intervention type	Outcome type	Study 1. First author 2. Year 3. Country	Setting 1. Specialty 2. Participant numbers	Participants: comparison 1. Age (years (SD)) 2. Gender (%Female) 3. BMI (mean, SD)	Participants: intervention 1. Age (years (SD)) 2. Gender (%Female) 3. BMI (mean, SD)	Comparison Usual care or placebo	Measures and timing 1. Measure 2. Baseline timing 3. Post-intervention	Comparison outcomes		Intervention 1. Name 2. Regimen, duration, how delivered	Intervention outcomes		SMD of Δ	Between group and time statistical significance
								1. Baseline (mean, SD) 2. Post-intervention (mean, SD)	3. Mean change, SD 4. Within group statistical significance		1. Baseline (mean, SD) 2. Post-intervention (mean, SD)	3. Mean change, SD 4. Within group statistical significance		
Physical activity	Physical performance	1.Wnuk 2. 2016 3. Poland	1. Vascular 2. Control n=16 Intervention n=15	1. 69 (4) 2. 0 3. 26.3 (3.5)	1. 68 (3) 2. 0 3. 26.2 (3.5)	Usual care (basic physiotherapy)	1. 6MWT (m) 2. Pre-operative 3. One week postoperative	1. 324.2 (63.4) 2. 258.1 (60.4)	3. -66.1 (45.6)	1. Backward walking 2. Steadily increased frequency for first 3 days then increased duration.	1. 362.3 (41.7) 2. 322.4 (64.7)	3. -39.9	+0.6	p=0.029 **Favours experimental**
		1.Wnuk 2. 2016 3. Poland	1. Vascular 2. Control n=16 Intervention n=16	1. 69 (4) 2. 0 3. 26.3 (3.5)	1. 70 (3) 2. 0 3. 26.6 (2.5)	Usual care (basic physiotherapy)	1. 6MWT (m) 2. Pre-operative 3. One week postoperative	1. 324.2 (63.4) 2. 258.1 (60.4)	3. -66.1 (45.6)	1. Forward walking 2. Steadily increased frequency for first 3 days then increased duration.	1. 338.3 (70.8) 2. 304.3 (73.0)	3. -34.0	+0.7	p=0.130 Favours null

		1. Sherrington 2. 2003 3. Australia	1. Orthopaedic rehabilitation 2. Control n=39 Intervention n=41	1. 81.1 (8.3) 2. 69 3. Unknown	1. 81.0 (7.0) 2. 66 3. Unknown	Non-weight-bearing exercise	1. Gait speed (m/s) 2. After randomisation 3. Two weeks after baseline	1. 0.09 (0.09) 2. 0.19 (0.20)	3. +0.10 (0.17)	1. Weight-bearing exercise 2. Individually progressed stepping exercises – increased repetitions, lessening hand support, increasing height of blocks.	1. 0.12 (0.10) 2. 0.25 (0.22)	3. +0.13	+0.2	p=0.69 Favours null
		1. Rahmann 2. 2009 3. Australia	1. Elective orthopaedic 2. Control n=20 Intervention n=24	1. 70.4 (9.2) 2. 70.6 3 28.8 (6.2)	1. 69.4 (6.5) 2. 44.4 3. 28.4 (4.6)	Ward-based physiotherapy	1. TUG (s) 2. Pre-operative 3. Two weeks postoperative	1. 15.5 (6.8) 2. 25.4 (14.4)	3. +9.9 (9.7)	1. Aquatic physiotherapy 2. Progressive trunk stability, backstroke kick, arm swing exercises in water	1. 12.3 (3.3) 2. 18.4 (10.1)	3. +6.2	+0.4	p=0.092 Favours null
		1. Rahmann 2. 2009 3. Australia	1. Elective orthopaedic 2. Control n=24 Intervention n=21	1. 69.4 (6.5) 2. 44.4 3. 28.4 (4.6)	1. 69.0 (8.9) 2. 43.2 3. 28.0 (4.1)	Aquatic physiotherapy	1. TUG (s) 2. Pre-operative 3. Two weeks postoperative	1. 12.3 (3.3) 2. 18.4 (10.1)	3. +6.2 (7.5)	1. Water exercise 2. Progressive trunk stability, backstroke kick, arm swing exercises in water	1. 16.4 (9.7) 2. 19.7 (6.7)	3. +3.3	+0.4	p=0.798 Favours null
		1. de Morton 2. 2007 3. Australia	1. General medicine 2. Control n=126 Intervention n=110	1. 78 (7) 2. 54 3. Unknown	1. 80 (8) 2. 55.5 3. Unknown	Usual care	1. TUG (s) 2. Within 48 hours of admission 3. At discharge	1. 30 (28) 2. 26 (21)	3. -5 (10)	1. Physiotherapy-designed exercises 2. Individualised progressive exercise training – lower limb, upper limb, and trunk.	1. 35 (30) 2. 36 (65)	3. -10 (19)	+0.5	p=0.63 Favours null

		1. Opasich 2. 2010 3. Italy	1. Cardiac surgery 2. Control n=80 Intervention n=160	1. 75.0 (3.9) 2. 45 3. Unknown	1. 74.6 (3.6) 2. 40 3. Unknown	Traditional physiotherapy (including bicycle, treadmill options)	1. a. TUG (s), b. 6MWT (m) 2. Within 2 days of admission 3. At discharge	1. a. 14.3 (6.2) b. 195.2 (86) 2. a. 11.1 b. 309.2	3. a. -3.2 (4) b. +114.0 (88) 4. a p<0.001 b. p<0.001	1. Individualised physical training programme 2. Exercises stratified by frailty ranging from assisted walking to treadmill and bicycle use.	1. a. 13.6 (6.1) b. 218.0 (92) 2. a. 8.8 b. 336.5	3. a. -4.8 (5.3) b. +118.5 (80) 4. a p<0.001 b. p<0.001	a. +0.4 b. -0.1	a. p<0.001 **Favours experimental** b. p=0.65 Favours null
		1. Schwenk 2. 2014 3. Germany	1. Geriatric rehabilitation 2. Control n=74 Intervention n=74	1. 83.9 (6.1) 2. 76.2 3. Unknown	1. 84.2 (6.2) 2. 83.6 3. Unknown	Usual care	1. Gait speed (m/s) 2. 1-2 days after admission 3. 1-2 days before discharge	1. 0.73 (0.39) 2. 0.89 (0.37)	3. +34.9% (53.8) 4. p<0.001	1. Individualised physical training programme 2. Progressively increased machine-based resistance, balance, functional exercises.	1. 0.73 (0.39) 2. 0.93 (0.38)	3. +43.3% (53.7) 4. p<0.001	+0.2	p=0.354 Favours null
		1. Giangregorio 2. 2009 3. Canada	1. Orthopaedic rehabilitation 2. Control n=7 Intervention n=14	1. 83.7 (8.6) 2. 85.7 3. Unknown	1. 79.9 (7.0) 2. 85.7 3. Unknown	Usual care	1. TUG (s) 2. Following recruitment 3. At discharge	1. 45.6 (10.3) 2. 20.7 (5.0)	3. -24.9 (6.9)	1. Body weight supported treadmill training 2. Treadmill and suspension system with progressively increased duration.	1. 73.5 (29.1) 2. 25.2 (29.1)	3. -48.3	+4.4	p=0.32 Favours null

		1. Zinglersen 2. 2018 3. Denmark	1. Geriatric medicine 2. Control n=48 Intervention n=20	1. 83.3 (8.1) 2. 75 3. 25.8 (5.3)	1. 84.9 2. 75 3. 25.6 (4.7)	Historical control group (usual care)	1. Gait speed (m/s) 2. Within 2 days of admission 3. Day of discharge or maximum 10 days	1. 0.60 (0.20) 2. 0.65	3. +0.05 4. p=0.36	1. Chair based functional training 2. Individualised training – progressively increased repetitions of chair stands, reduced height of blocks (with or without NMES).	1. 0.50 (0.20) 2. 0.61	3. +0.11 4. p<0.01	NA	Not statistically different Favours null
		1. Moseley 2. 2009 3. Australia	1. Orthopaedic rehabilitation 2. Control n=80 Intervention n=80	1. 84 (8) 2. 81.3 3. 23.4	1. 84 (8) 2. 81.3 3. 24.0	Low dose (limited) weight-bearing exercise	1. Gait speed (m/s) 2. Recruitment 3. Four weeks follow-up	1. 0.28 (0.16) 2. 0.48 (0.22)	3. +0.20 (0.14)	1. Weight-bearing exercise 2. Progressive reduced support (harness whilst inpatient), increased repetition	1. 0.30 (0.22) 2. 0.53 (0.25)	3. +0.23	+0.2	p=0.345 Favours null
		1. Busch 2. 2012 3. Germany	1. Cardiac surgery 2. Control n=64 Intervention n=57	1. 78.6 (3.2) 2. 71 3. 26.8	1. 78.5 (3.2) 2. 67 3. 27.9	Usual care (thrice weekly walks, calisthenics, ergometer)	1. a. TUG (s) b. 6MWT (m) 2. Before rehabilitation 3. At discharge	1. a. 10 (3) b. 311 (80) 2. a. 9 (4) b. 352 (82)	3. a. -1 (3) b. +42 (52) 4. a. p<0.001	1. Resistance and balance training 2. Weight machines for lower limb exercises, free weights for upper limb; balls and platforms for balance	1. a. 11 (3) b. 296 (84) 2. a. 8 (2) b. 363 (86)	3. a. -2 (2) b. +67 (49) 4. a. p<0001	a. +0.3 b. +0.5	a. p=0.003 **Favours experimental** b. p=0.008 **Favours experimental**

		1. Raymond 2. 2017 3. Australia	1. Geriatric medicine 2. Control n=232 Intervention n=236	1. 84.1 (6.9) 2. 57.8 3. Unknown	1. 84.5 2. 63.1 3. Unknown	Usual care (physiotherapy 5 days/ week)	1. TUG(s) 2. Prior to randomisation 3. Within 48 hours prior to discharge	1. 29 (95% CI 23-42) 2. 24 (95% CI 17-34)	3. -5	1. High intensity group exercise 2. Group classes thrice weekly focussing on progressive resistance, balance exercise	1. 29 (95% CI 20-42) 2. 22 (95% CI 16-33)	3. -7	NA	p=0.47 Favours null
		1. McCullagh 2. 2017 3. Ireland	1. General medicine 2. Control n=95 Intervention n=95	1. 81.7 (7.3) 2. 41 3. 26.8 (6.8)	1. 79.7 (7.5) 2. 64 3. 26.3 (6.5)	Sham exercise programme (gentle stretching and relaxation)	1. a. SPPB b. Gait speed (m/s)[b] 2. Within 48 hours of admission 3. Within 24 hours of discharge	1. a. 3 (95% CI 2 to 4) b. 0.32 (0.18) 2. a. 3.0 (2.1) b. 0.30 (0.20)	3. a. +0 b. -0.02 (0.20)	1. Augmented prescribed exercise programme 2. Individually progressed lower limb and core strengthening exercises.	1. a. 3 (95% CI 2 to 5) b. 0.30 (0.18) 2. a. 4.6 (2.5) b. 0.25 (0.14)	3. a. +1.6 b. -0.05	a. NA b. -0.2	a. p=0.003 **Favours experimental** b. Favours null
		1. Martinez-Velilla 2. 2019 3. Spain	1. Geriatric medicine 2. Control n=185 Intervention n=185	1. 87.1 (5.2) 2. 58.9 3. 26.9 (4.9)	1. 87.6 (4.6) 2. 54.1 3. 27.1 (4.4)	Usual care	1. a. Gait speed (m/s)[b] b. SPPB 2. Start of intervention 3. At discharge	1. a.0.46 (0.20) b. 4.7 (2.7) 2. a. 0.48 (0.19) b. 4.9	3. a.+0.01 (0.12) b. +0.2 (95% CI -0.1 to 0.5)	1. Multicomponent physical exercise 2. Individualised progressive resistance (machines, weights), balance, walking exercises.	1. a.0.48 (0.19) b. 4.4 (2.5) 2. a. 0.61 (0.22) b. 6.8	3. a.+0.12 (0.13) b. +2.4 (95% CI 2.1 to 2.7)	a. +0.9 b. NA	a. p<0.001 **Favours experimental** b. p<0.001 **Favours experimental**

		1. Jones 2. 2006 3. Australia	1. General medicine 2. Control n=80 Intervention n=80	1. 82.9 (7.6) 2. 61.3 3. Unknown	1. 81.9 (8.0) 2. 53.8 3. Unknown	Usual care	1. TUG (s) 2. Within 48 hours of admission 3. Within 24 hours of discharge	1. 21.5 (95% CI 16.9 to 25.9) 2. 20.3	3. -1.2 (95% CI -0.9 to 4.3) 4. p=0.012	1. Individualised progressive exercise 2. Strengthening and mobility exercise ranging from bed to stairs exercise.	1. 24.2 (95% CI 15.8 to 37.3) 2. 18.8	3. -5.4 (95% CI -1.0 to -12.4) 4. p=0.012	NA	p=0.081 Favours null
		1. Fiore[b] 2. 2017 3. Canada	1. Elective colorectal surgery 2. Control n=22 Intervention= 25	1. 73.1 (5.9) 2. 54.5 3. Unknown	1.72.8 (6.5) 2. 40 3. Unknown	Usual care (enhanced recovery after surgery)	1. 6MWT (m) 2. Pre-operative 3. Four weeks post-operative	1. 487.0 (71.6) 2. 439.5 (103.3)	3. -47.6 (88.0) 4. p=0.034	1. Early mobilisation 2. Physiotherapy/ physiotherapy-trained facilitated mobilisation – thrice day review	1. 409.0 (117.6) 2. 362.4 (174.8)	3. -46.6 (116.2) 4. p=0.067	+0.0	p=0.977 Favours null
		1. Tal-Akabi[b] 2. 2007 3. Switzerland	1. Orthopaedic rehabilitation 2. Control n=29 Intervention n=33	1. 74.6 (7.8) 2. 75.9 3. 25.4 (6.3)	1. 73.7 (6.0) 2. 66.7 3. 26.8 (5.3)	Regular intensity exercise	1.TUG (s) 2. Within 48 hours of admission 3. Within 24 hours before discharge	1. 29.6 2. 17.6	3.-8 4. p<0.001	1. High intensity exercise 2. Individually progressive leg press functional exercise.	1. 27.6 2. 15.9	3.-11.7 4. p<0.001	NA	Not statistically different Favours null
		1. Houborg 2. 2006 3. Denmark	1. Elective colorectal surgery 2. Control n=59 Intervention n=60	1. 72 (7) 2. 49.2 3. 26 (3)	1. 72 (7) 2. 50 3. 26 (5)	Sham treatment – turning, repositioning, relaxation, massages	1. Gait speed (m/s) 2. Pre-operative 3. 7 days post-operative	1. 1.48 (0.31) 2. 1.13	3. -0.35 (95% CI -0.28 to -0.47) 4. p=0.98	1. Strength training programme 2. Progressive strength training of upper and lower limbs, mobilisation, aerobic training.	1. 1.39 (0.38) 2. 1.04	3. -0.35 (95% CI -0.25 to -0.5) 4. p=0.98	NA	Not statistically different Favours null

		1. Said [b] 2. 2018 3. Australia	1. Geriatric rehabilitation 2. Control n=93 Intervention n=98	1. 81 (95% CI 77 to 87) 2. 55 3. 25.0 (95% CI 22.5 to 28.9)	1. 81 (95% CI 77 to 88) 2. 60 3. 24.3 (95% CI 20.9 to 29.2)	Usual care and additional social activities not impacting on mobility	1. a. Gait speed (m/s) b. TUG (s) 2. Within 48 hours of admission 3. Within 48 hours of discharge	1. a. 0.29 (0.26) b. 33.8 (19.4) 2. a. 0.56 (0.28) b. 26.2 (20.5)	3. a.+0.27 (0.18) b. -7.6 (14.7)	1. Multimodal exercise programme 2. Progressive functional, balance, strength, mobility and aerobic training.	1. a. +0.31 (0.28) b. 31.5 (18.5) 2. a. 0.51 (0.29) b. 24.2 (12.5)	3. a. +0.20 b. -7.3	a. -0.4 b. +0.0	a. p=0.096 Favours null b. p=0.724 Favours null
		1. Sano 2. 2018 3. Spain	1. Elective orthopaedic 2. Control n=41 Intervention n=40	1. 75 (5.8) 2. 78.9 3. 26.6 (3.2)	1. 75 (6.4) 2. 81.1 3. 25.9 (3.5)	Usual care (physical therapy 5 days/week for 3 weeks)	1. a. Gait speed (m/s) b. TUG (s) 2. Pre-operative 3. Three weeks post-operative	1. a. 1.02 (0.21) b. 10.81 (2.71) 2. a. 0.92 (0.22) b. 12.22 (3.15)	3. a. -0.10 (0.13) b. +1.41 (1.85)	1. Seated side tapping training 2. Five repetitions tapping outstretched arms 10 times.	1. a. 1.02 (0.21) b. 10.94 (2.59) 2. a. 1.03 (0.19) b. 10.44 (1.87)	3. a. +0.01 b. -0.50	a. +0.8 b. +1.0	a. p=0.001 **Favours experimental** b. p=0.001 **Favours experimental**
		1. Said [b] 2. 2012 3. Australia	1. Geriatric rehabilitation 2. Control n=24 Intervention n=22	1. 81.6 (6.5) 2. 40 3. Unknown	1. 80.8 (4.6) 2. 59 3. Unknown	Usual care	1. TUG (s) 2. Within 48 hours of admission 3. Within 48 hours of discharge	1. 31.3 (12.4) 2. 32.6 (17.4)	3. +1.3 (10.3)	1. Enhanced physical activity 2. Increasing mobility activities evenings and weekends.	1. 35.5 (11.8) 2. 36.8 (26.7)	3. +1.3	0	Not statistically different Favours null

		1. Prasciene 2. 2019 3. Lithuania	1. Cardiac surgery 2. Control n=15 Intervention n=14	1. 76.5 (2.1) 2. 60 3. Unknown	1. 72.8 (2.0) 2. 40 3. Unknown	Standard care – comprehensive three week exercise-based rehabilitation	1. a. SPPB b. 6MWT 2. Before rehabilitation 3. After rehabilitation	1. a. 7.5 (0.6) b. 242.3 (32.1) 2. a. 8.4 (0.7) b. 341.8 (30.3)	3. a. +0.9 b. +99.5 (23.0)	1. Additional exercise 2. Additional exercise session three days/week including balance and resistance training	1. a. 8.4 (0.6) b. 239.3 (28.6) 2. a. 9.6 (0.7) b. 359.0 (27.1)	3. a. +1.2 b. +119.7 (20.5)	a. NA b. +0.9	a. p=0.202 Favours null b. p=0.677 Favours null
		1. Ortiz-Alonso 2. 2019 3. Spain	1. Geriatric medicine 2. Control n=131 Intervention n=150	1. 88 (5) 2. 54 3. 26.0 (6.4)	1. 88 (5) 2. 60 3. 26.1 (9.3)	Usual care	1. SPPB 2. Admission 3. Discharge	1. 3.8 (2.9) 2. 4.1	3. +0.3 (2.2)	1. Exercise programme 2. Chair rises and walking	1. 3.2 (2.5) 2. 3.6	3. +0.4 (1.8)	+0.0	p=0.796 Favours null
		1. Deer 2. 2019 3. USA	1. General Medicine 2. Control n=20 3. Intervention n=21	1. 75.7 (7.1) 2. 70 3. 29.0 (5.3)	1. 77.6 (7.5) 2. 67 3. 27.4 (6.4)	Placebo isocaloric supplement, usual care	1. SPPB 2. During hospitalisation 3. Four weeks follow-up	1. 7.8 (3.7) 2. 9.1	3. +1.3 (1.9)	1. Rehabilitation 2. Chair-based exercises and resistance exercise	1. 7.1 (2.9) 2. 9.1	3. +2.0 (1.9)	+0.4	p=0.03 *(all interventions vs. placebo)* **Favours experimental**

		1. Braun 2. 2019 3. Germany	1. Geriatric medicine 2.Control n=18 Intervention n=17	1. 83.1 (7.4) 2. 72 3. Unknown	1. 78.6 2. 76 3. Unknown	Usual care	1. a. Gait speed (m/s) b. TUG (s) c. 6MWT (m) 2. After randomisation 3. 14 days after admission	1. a. 0.60 (0.19) b. 24.9 (11.1) c. 167.7 (79.4) 2. a. 0.64 (0.28) b. 22.4 (9.5) c. 170.8 (79.9)	3. a. +0.04 (0.15) b. -2.5 (5.9) c. +3.1 (37.7)	1. Augmented Prescribed Exercise Program 2. Individually tailored exercises from chair-based to endurance and/or walking	1. a. 0.53 (0.17) b. 28.6 (13.2) c. 154.5 (59.6) 2. a. 0.65 (0.20) b. 22.8 (12.2) c. 194.9 (85.8)	3. a. +0.12 (0.20) b. -5.8 (6.6) c. +40.4 (80.9)	a. +0.5 b. +0.6 c. +1.0	a. p=0.25 Favours null b. p=0.21 Favours null c. p=0.11 Favours null
	Muscle strength	1. Sherrington 2. 2003 3. Australia	1. Orthopaedic rehabilitation 2. Control n=39 Intervention n=41	1. 81.1 (8.3) 2. 69 3. Unknown	1. 81.0 (7.0) 2. 66 3. Unknown	Non-weight-bearing exercise	1. Knee extension (kg) 2. After randomisation 3. Two weeks after baseline	1. 94.9 (44.1) 2. 109.1 (50.8)	3. +14.2 (32.8)	1. Weight-bearing exercise 2. Individually progressed stepping exercises – increased repetitions, lessening hand support, increasing height of blocks.	1. 112.0 (63.1) 2. 118.7 (61.7)	3. +6.7	-0.2	p=0.14 Favours null
		1. Rahmann 2. 2009 3. Australia	1. Elective orthopaedic 2. Control n=20 Intervention n=24	1. 70.4 (9.2) 2. 70.6 3 28.8 (6.2)	1. 69.4 (6.5) 2. 44.4 3. 28.4 (4.6)	Ward-based physiotherapy	1. Knee extension (kg) 2. Preoperative 3. Two weeks postoperative	1. 10.1 (4.8) 2. 8.6 (4.6)	3. -1.5 (3.2)	1. Aquatic physiotherapy 2. Progressive trunk stability, backstroke kick, arm swing exercises in water	1. 14.8 (6.2) 2. 12.2 (4.2)	3. -2.6	-0.3	p=0.030 Favours control

		1. Rahmann 2. 2009 3. Australia	1. Elective orthopaedic 2. Control n=24 Intervention n=21	1. 69.4 (6.5) 2. 44.4 3. 28.4 (4.6)	1. 69.0 (8.9) 2. 43.2 3. 28.0 (4.1)	Aquatic physiotherapy	1. Knee extension (kg) 2. Pre-operative 3. Two weeks postoperative	1. 14.8 (6.2) 2. 12.2 (4.2)	3. -2.6 (4.0)	1. Water exercise 2. Progressive trunk stability, backstroke kick, arm swing exercises in water	1. 11.5 (6.0) 2. 9.7 (3.7)	3. -1.8	+0.2	p=0.456 Favours null
		1. Schwenk 2. 2014 3. Germany	1. Geriatric rehabilitation 2. Control n=74 Intervention n=74	1. 83.9 (6.1) 2. 76.2 3. Unknown	1. 84.2 (6.2) 2. 83.6 3. Unknown	Usual care	1. Handgrip (kg) 2. 1-2 days after admission 3. 1-2 days before discharge	1. 14.6 (6.2) 2. 15.1 (6.6)	3. +5.7% (21.0) 4. p=0.084	1. Individualised physical training programme 2. Progressively increased machine-based resistance, balance, functional exercises	1. 14.6 (6.2) 2. 14.8 (6.7)	3. +6.6% (43.9) 4. p=0.084	+0.0	p=0.834 Favours null
		1. Torres-Sánchez 2. 2017 3. Spain	1. Respiratory 2. Control n=29 Intervention n=29	1. 72.2 (8.2) 2. 31.0 3. 29.1 (2.5)	1.76.7 (6.3) 2. 24.1 3. 31.3 (1.8)	Usual care	1. Knee extension (kg) 2. Second day of admission 3. Day of discharge	1. 10.3 (1.4) 2. 8.8 (4.1)	3. -1.5 (3.8)	1. Pedal exercises 2. Individualised progressive time, velocity, and resistance of pedal exercises.	1. 10.6 (11.2) 2. 11.6 (3.8)	3. +1.0	+0.7	p=0.028 **Favours experimental**
		1. Moseley 2. 2009 3. Australia	1. Orthopaedic rehabilitation 2. Control n=80 Intervention n=80	1. 84 (8) 2. 81.3 3. 23.4	1. 84 (8) 2. 81.3 3. 24.0	Low dose (limited) weight-bearing exercise	1. Knee extension (kg) 2. Recruitment 3. Four weeks follow-up	1. 6.8 (3.4) 2. 7.7 (4.0)	3. +0.9 (2.6)	1. Weight-bearing exercise 2. Progressive reduced support (harness whilst inpatient), increased repetition	1. 7.4 (3.3) 2. 7.8 (3.9)	3. +0.4	-0.2	p=0.853 Favours null

		1. Busch 2. 2012 3. Germany	1. Cardiac surgery 2. Control n=64 Intervention n=57	1. 78.6 (3.2) 2. 71 3. 26.8	1. 78.5 (3.2) 2. 67 3. 27.9	Usual care (thrice weekly walks, calisthenics, ergometer)	1. Knee extension (kg) 2. Before rehabilitation 3. At discharge	1. 30.2 (12.0) 2. 34.7 (14.8)	3. +4.5 (9.5) 4. p<0.001	1. Resistance and balance training 2. Weight machines for lower limb exercises, free weights for upper limb; balls and platforms for balance	1. 33.8 (13.3) 2. 39.6 (16.0)	3. +5.8 4. p<0.001	+0.1	p=0.49 Favours null
		1. Martinez-Velilla 2. 2019 3. Spain	1. Geriatric medicine 2. Control n=185 Intervention n=185	1. 87.1 (5.2) 2. 58.9 3. 26.9 (4.9)	1. 87.6 (4.6) 2. 54.1 3. 27.1 (4.4)	Usual care	1. Handgrip (kg) 2. Start of intervention 3. At discharge	1. 17.0 (8.0) 2. 16.2	3. -0.8 (95% CI -1.2 to -0.5; SD 2.4)	1. Multi-component physical exercise 2. Individualised progressive resistance (machines, weights), balance, walking exercises.	1. 17 (6) 2. 18.5	3. +1.5 (95% CI 1.1 to 1.8)	+1.0	p<0.001 **Favours experimental**
		1. Blanc-Bisson[b] 2.2008 3.France	1. Geriatric medicine 2. Control n=24 Intervention n=22	1. 83.9 (6.6) 2. 78.9 3. 22.9 (4.7)	1. 86.6 (5.0) 2. 65.8 3. 25.1 (5.4)	Usual care	1. Handgrip strength (kg) 2. At recruitment 3.'Clinical stability' – mean 12.4 (4.7) days	1. 15.8 (8.6) 2. 16.8 (7.6)	3.+0.6 (4.7)	1. Early physiotherapy 2. Progressive exercise in bed then upright when able to stand.	1. 16.1 (6.9) 2. 16.8 (8.0)	3. +1.0 (3.4)	+0.1	p=0.753 Favours null

		1. Henriksen[b] 2. 2002 3. Denmark	1. Elective colorectal surgery 2. Control n=12 Intervention n=13	1. 74.4 (4.0) 2. 33.3 3. Unknown	1. 73.5 (5.5) 2. 69.2 3. Unknown	Normal mobilisation by nursing staff	1. a. Handgrip strength (kg) b. Knee extension (kg) 2. Pre-operative 3. 7 days post-operative	1. a. 38.1 (12.9) b. 35.2 (10.4) 2. a. 38.1 (12.9) b. 33.9 (10.1)	3. a. -2.8 (3.4) b. -5.2 (3.9)	1. Enhanced recovery 2. Early mobilisation	1. a. 28.9 (10.2) b. 22.9 (9.9) 2. a. 28.3 (10.0) b. 22.7 (8.3)	3. a. -0.7 (1.1) b. -0.2 (4.0)	a. +0.6 b. +1.3	a. p=0.100 Favours null b. p=0.042 **Favours experimental**
		1. Houborg 2. 2006 3. Denmark	1. Elective colorectal surgery 2. Control n=59 Intervention n=60	1. 72 (7) 2. 49.2 3. 26 (3)	1. 72 (7) 2. 50 3. 26 (5)	Sham treatment – turning, repositioning, relaxation, massages	1. a. Knee extension (kg) b. Handgrip (kg) 2. Pre-operative 3. 7 days post-operative	1. a. 30.0 (12.5) b. 30.4 (13.0) 2. a. 23.4 b. 28.4	3. a. -6.6 (95% CI -4.1 to 9.2) b. -2.0 (95% CI 0 to -3.6) 4. a. p=0.14 b. p=0.42	1. Strength training programme 2. Progressive strength training of upper and lower limbs, mobilisation, aerobic training.	1. a.28.2 (12.0) b. 29.1 (12.2) 2. a. 24.1 b.	3. a. -4.1 (95% CI -1.5 to -6.6) b. -3.1 (95% CI -1.0 to 5.1) 4. a. p=0.14 b. p=0.42	NA	a. Not statistically different Favours null b. Not statistically different Favours null
		1.McGowan[b] 2. 2018 3. UK	1. Acute medicine for older people 2. Control n=25 Intervention n=25	1.82.9 (5.7) 2. 54.2 3. Unknown	1. 87.1 (9.2) 2. 66.7 3. Unknown	Usual care	1. a. Knee extension (kg) b. Knee flexion (kg) 2. Within 48 hours of admission 3. At 7 days or discharge if earlier.	1. a. 8.9 (2.7) b. 9.6 (2.9) 2. a. 9.0 (3.5) b. 9.2 (2.7)	3. a. -0.4 (2.7) b. +0.1 (3.2) 4. a. p=0.887 b. p=0.321	1. Pedal exerciser 2. Thrice daily pedal exercises, five minutes each.	1. a. 8.1 (2.4) b. 9.8 (2.9)	3. a. +0.3 (2.2) b. +0.2 (2.1) 4. a. p=0.588 b. p=0.714	a. +0.3 b. +0.0	a. p=0.851 Favours null b. p=309 Favours null

		1. McCullagh 2. 2017 3. Ireland	1. General medicine 2. Control n=95 Intervention n=95	1. 81.7 (7.3) 2. 41 3. 26.8 (6.8)	1. 79.7 (7.5) 2. 64 3. 26.3 (6.5)	Sham exercise programme (gentle stretching and relaxation)	1. Handgrip (kg) 2. Within 48 hours of admission 3. Within 24 hours of discharge	1. 17.0 (7.8) 2. 18.1 (7.0)	3. +1.1 (2.9)	1. Augmented prescribed exercise programme 2. Individually progressed lower limb and core strengthening exercises.	1. 16.9 (7.6) 2. 18.1 (7.4)	3. +1.2	+0.0	Favours null
		1. Sano 2. 2018 3. Spain	1. Elective orthopaedic 2. Control n=41 Intervention n=40	1. 75 (5.8) 2. 78.9 3. 26.6 (3.2)	1. 75 (6.4) 2. 81.1 3. 25.9 (3.5)	Usual care (physical therapy 5 days/week for 3 weeks)	1. a. Knee extension (kg) b. Knee flexion (kg) 2. Pre-operative 3. Three weeks post-operative	1. a. 16.0 (6.7) b. 9.2 (4.5) 2. a. 7.8 (3.3) b. 6.9 (2.4)	3. a. -8.2 (4.7) b. -2.3 (3.7)	1. Seated side tapping training 2. Five repetitions tapping outstretched arms 10 times.	1. a. 16.2 (7.1) b. 9.5 (3.8) 2. a. 7.7 (2.9) b. 6.7 (2.4)	3. a. -8.5 b. -2.8	a. -0.1 b. -0.1	a. p=0.883 Favours null b. p=0.654 Favours null
	Muscle mass	1. Deer 2. 2019 3. USA	1. General Medicine 2. Control n=20 3. Intervention n=21	1. 75.7 (7.1) 2. 70 3. 29.0 (5.3)	1. 77.6 (7.5) 2. 67 3. 27.4 (6.4)	Placebo isocaloric supplement, usual care	1. DXA FFM (kg) 2. During hospitalisation 3. Four weeks follow-up	1. 44.6 (9.8) 2. 45.1	3. +0.5 (0.9)	1. Rehabilitation 2. Chair-based exercises and resistance exercise	1. 42.7 (9.8) 2. 43.6	3. +0.9 (1.8)	+0.4	p=0.72 *(all interventions vs. placebo)* Favours null
Nutrition	Physical performance	1. Niccoli 2. 2017 3. Canada	1. Geriatric medicine 2. Control n=26 Intervention n=26	1. 80.3(1.6) 2. 68 3. 26.4 (6.6)	1.81.8 (1.7) 2. 68.2 3. 24.2 (5.2)	Usual care	1. a. TUG (s) b. Gait speed (m/s) 2. At recruitment 3. Prior to discharge	1. a. 0.56 (0.06) b. 28.2 (2.8) 2. a. 0.74 (0.06) b. 21.8 (2.2)	3. a. +0.18 (0.03) b. -6.4 (1.9) 4. a. p<0.001 b. p=0.002	1. Whey protein 2. Whey protein mixed into cereal and/or milk products.	1. a. 0.52 (0.03) b. 28.3 (3.1) 2. a. 0.66 (0.04) b. 21.2 (2.0)	3. a. +0.15 (0.04) b. -7.1 (2.2) 4. a. p<0.001 b. p=0.003	a. +1.0 b. +0.4	Not statistically different Favours null

		1. Beelen 2. 2017 3. Netherlands	1. General medicine 2. Control n=39 Intervention n=36	1. 77.2 (7.2) 2. 56.4 3. 28.2 (5.6)	1. 76.5 (6.7) 2. 55.6 3. 26.9 (6.1)	Regular non-enriched variants of intervention products	1. SPPB 2. Within 2 days of hospital admission 3. Two weeks post-discharge	1. 6.0 (2.5) 2. 6.9 (2.5)	3. +0.9	1. Protein-enriched familiar foods 2. Patients could order food choices from menu without knowledge of enrichment.	1. 6.8 (0.4) 2. 7.2 (0.5)	3. +0.4	NA	Not statistically different Favours null
		1. Gade 2. 2019 3. Denmark	1. General medicine 2. Control n=82 Intervention n=83	1. 84.2 (6.3) 2. 65.3 3. 25.8 (5.2)	1. 85.3 (6.2) 2. 69.9 3. 25.1 (4.2)	Placebo isoenergetic beverage, resistance exercise, vitamin D	1. Gait speed (m/s) 2. Within 72 hours of admission 3. Within 72 hours of discharge	1. 0.5 (IQR 0.4 -0.7) 2. 0.5	3. +0.0 (IQR -0.1 – 0.7)	1. Protein-enriched milk-based supplement 2. After breakfast and resistance exercise, vitamin D	1. 0.6 (IQR 0.5 – 0.9) 2. 0.6	3. +0.0 (IQR -0.1 – 0.1)	NA	p=0.481 Favours null
		1. Pedersen 2. 2019 3. Denmark	1. General medicine 2. Control n=42 Intervention n=43	1. 82.5 (7.5) 2. 60.5 3. 24.5 (95% CI 22.3 – 30.0)	1. 82.1 (7.4) 2. 71.4 3. 25.3 (95% CI 22.3 – 29.1)	Standard care	1. Gait speed (m/s) 2. On admission 3. Within first week after discharge	1. 0.6 (95% CI 0.5 – 0.8) 2. 0.7 (95% CI 0.5 – 0.8)	3. +0.1	1. Protein and exercise 2. Progressive strength training and immediate protein supplementation	1. 0.6 (95% CI 0.4 – 0.8) 2. 0.7 (95% CI 0.5 – 0.9)	3. +0.1	NA	p=0.06 Favours null
		1. Deer 2. 2019 3. USA	1. General Medicine 2. Control n=20 3. Intervention n=20	1. 75.7 (7.1) 2. 70 3. 29.0 (5.3)	1. 80.0 (8.7) 2. 70 3. 28.9 (6.4)	Placebo isocaloric supplement, usual care	1. SPPB 2. During hospitalisation 3. Four weeks follow-up	1. 7.8 (3.7) 2. 9.1	3. +1.3 (1.9)	1. Whey protein 2. Twice daily 20g whey protein	1. 6.2 (3.1) 2. 8.9	3. +2.7 (2.3)	+0.7	p=0.03 *(all interventions vs. placebo)* **Favours experimental**

		1. Deer 2. 2019 3. USA	1. General Medicine 2. Control n=20 3. Intervention n=20	1. 75.7 (7.1) 2. 70 3. 29.0 (5.3)	1. 80.0 (8.8) 2. 70 3. 26.1 (6.6)	Placebo isocaloric supplement, usual care	1. SPPB 2. During hospitalisation 3. Four weeks follow-up	1. 7.8 (3.7) 2. 9.1	3. +1.3 (1.9)	1. Whey protein and rehabilitation 2. Chair-based exercises and resistance exercise and whey protein	1. 6.2 (3.5) 2. 9.6	3. +3.4 (2.1)	+1.1	p=0.03 *(all interventions vs. placebo)* **Favours experimental**
		1. Files 2. 2020 3. USA	1. Critical care 2. Control n=11 Intervention n=11	1. 67.9 (2.6) 2. 45.5 3. 31.3 (1.7)	1. 69.1 (3.2) 2. 45.5 3. 30.6 (1.9)	Placebo nitrate-depleted beetroot juice	1. SPPB 2. ICU discharge 3. Hospital discharge or 14 days after visit 1	1. 0 (IQR 0 – 2) 2. 3 (IQR 2 – 6)	3. +3	1. Nitrate-rich beetroot juice 2. Once/ day for 14 days	1. 0 (IQR 0 – 0.5) 2. 0 (IQR 0 – 5.5)	3. +0.0	NA	p=0.14 Favours null
	Muscle strength	1. Beelen 2. 2017 3. Netherlands	1. General medicine 2. Control n=39 Intervention n=36	1. 77.2 (7.2) 2. 56.4 3. 28.2 (5.6)	1. 76.5 (6.7) 2. 55.6 3. 26.9 (6.1)	Regular non-enriched variants of intervention products	1. a. Handgrip (kg) b. Knee extension (kg) 2. Within 2 days of hospital admission 3. Two weeks post-discharge	1. a. 25.5 (12.5) b. 22.2 (4.4) 2. a. 24.6 (8.1) b. 22.7 (4.8)	3. a. -0.9 (5.8) b. +0.5 (3.1)	1. Protein-enriched familiar foods 2. Patients could order food choices from menu without knowledge of enrichment.	1. a. 27.1 (9.6) b. 23.1 (4.3) 2. a. 25.3 (8.4) b. 24.5 (4.7)	3. a. -1.8 b. +2.4	a. -0.2 b. +0.3	Not statistically different Favours null
		1. Ekinci 2. 2016 3. Turkey	1. Orthopaedic surgery 2. Control n=37 Intervention n=38	1. 83.1 (7.1) 2. 100 3. 22.3 (2.7)	1. 82.2 (7.3) 2. 100 3. 21.8 (2.1)	Standard post-operative nutrition	1. Handgrip (kg) 2. Pre-operative 3. 15 days post-operative	1. 5.3 (3.4) 2. 6.3 (3.7)	3. +1.0 (0.4) 4. p=0.001	1. Beta-hydroxy-beta-methylbutyrate (HMB) supplementation 2. Two servings HMB enriched products in addition to standard nutrition	1. 7.1 (4.0) 2. 7.8 (4.1)	3. +0.7 4. p=0.001	-0.8	p=0.338 Favours null

		1. Hermanky 2. 2017 3. Austria	1. Orthopaedic surgery 2. Control n=20 Intervention n=20	1. 79.9 (8.5) 2. 65 3. 24.8 (4.1)	1. 79.1 (9.3) 2. 66.7 3. 26.3 (5.9)	Usual care (limited details)	1.Handgrip (kg) 2. Within 48 hours of admission 3. At discharge	1. 22.5 (8.4) 2. 21.6 (7.9)	3. -0.9 (3.1) 4. p=0.041	1. Nutrition and exercise 2. Nutritional consultation to reach defined energy and protein intake with moderate strength training.	1. 18.6 (6.2) 2. 18.9 (6.0)	3. -0.3 4. p=0.166	+0.2	p=0.570 Favours null
		1. Saudny-Unterberger 2. 1997 3. Canada	1. Respiratory 2. Control n=16 Intervention n=17	1. 69.4 (3.9) 2. 30 3. 25.7	1. 69.2 (2.2) 2. 43 3. 23.4	Food ordered from hospital menu	1. Handgrip (kg) 2. At admission 3. Two weeks post admission	1. 26.0 (2.8) 2. 26.4	3. +0.4 (0.9)	1. Oral nutritional supplements 2. Nutritional supplements and snacks between meals.	1. 29.7 (3.0) 2. 28.8	3. -0.9 (1.0)	-1.4	p=0.385 Favours null
		1. Niccoli 2. 2017 3. Canada	1. Geriatric medicine 2. Control n=26 Intervention n=26	1. 80.3(1.6) 2. 68 3. 26.4 (6.6)	1.81.8 (1.7) 2. 68.2 3. 24.2 (5.2)	Usual care	1. a. Handgrip (kg) b. Knee extension (kg) 2. At recruitment 3. Prior to discharge	1. a. 15.3 (1.4) b. 15.8 (1.0) 2. a. 15.8 (1.4) b. 17.3 (1.1)	3. a. +0.7 (0.5) b. +1.5 (0.4) 4. a. 0.244 b. p=0.170	1. Whey protein 2. Whey protein mixed into cereal and/or milk products.	1. a. 13.4 (1.2) b. 12.3 (1.6) 2. a. 15.0 (1.1) b. 15.2 (1.3)	3. a. +2.0 (0.8) b. +2.9 (1.8) 4. a. p<0.001 b. p=0.032	a. +2.6 b. +3.5	a. p=0.455 Favours null b. p=0.071 Favours null
		1. Gade 2. 2019 3. Denmark	1. General medicine 2. Control n=82 Intervention n=83	1. 84.2 (6.3) 2. 65.3 3. 25.8 (5.2)	1. 85.3 (6.2) 2. 69.9 3. 25.1 (4.2)	Placebo isoenergetic beverage, resistance exercise, vitamin D	1. Handgrip (kg) 2. Within 72 hours of admission 3. Within 72 hours of discharge	1. 17.7 (IQR 13.1 – 22.7) 2. 17.4	1. -0.3 (IQR -2.2 - +2.3)	1. Protein-enriched milk-based supplement 2. After breakfast and exercise	1. 17.8 (IQR 13.3 – 23.3) 2. 18.0	3. +0.2 (IQR-1.9 - +0.6)	NA	p=0.681 Favours null

		1. Pedersen 2. 2019 3. Denmark	1. General medicine 2. Control n=42 Intervention n=43	1. 82.5 (7.5) 2. 60.5 3. 24.5 (95% CI 22.3 – 30.0)	1. 82.1 (7.4) 2. 71.4 3. 25.3 (95% CI 22.3 – 29.1)	Standard care	1. Handgrip (kg) 2. On admission 3. Within first week after discharge	1. 21.1 (8.7) 2. 21.8 (8.9)	3. +0.7 (3.3)	1. Protein and exercise 2. Progressive strength training and immediate protein supplementation	1. 21.5 (10.3) 2. 23.5 (9.9)	3. +2.0 (3.8)	+0.4	p=0.008 **Favours experimental**
	Muscle mass	1. Hermanky 2. 2017 3. Austria	1. Orthopaedic surgery 2. Control n=20 Intervention n=20	1. 79.9 (8.5) 2. 65 3. 24.8 (4.1)	1. 79.1 (9.3) 2. 66.7 3. 26.3 (5.9)	Usual care (limited details)	1.Bioelectrical Impedance Analysis – Fat Free Mass (kg) 2. Within 48 hours of admission 3. At discharge	1. Unknown 2. Unknown	3. -1.347 4. p=0.162	1. Nutrition and exercise 2. Nutritional consultation to reach defined energy and protein intake with moderate strength training.	1. Unknown 2. Unknown	3. -0.324 4. p=0.626	NA	Favours null
		1. Ogasawara 2. 2018 3. Japan	1. Respiratory medicine 2. Control n=21 Intervention n=21	1. 79.1 (6.8) 2. 4.8 3. 19.1 (2.8)	1. 79.5 (8.1) 2. 14.3 3. 19.3 (2.4)	Similar energy oral nutritional supplements free of EPA	1. Bioelectrical Impedance Analysis – Skeletal Muscle Index (kg/m^2) 2. Admission 3. Discharge	1. 5.9 (1.0) 2. 5.6 (14)	3. +0.3 4. p=0.35	1. EPA-enriched oral nutritional supplements 2. One can or pack given each day	1. 6.0 (1.1) 2. 6.2 (1.1)	3.+ 0.2 4. p=0.13	NA	p=0.10 Favours null
		1. Bouillanne 2. 2018 3. France	1. Geriatric medicine rehabilitation 2. Control n=14 Intervention n=13	1. 88 (95% CI 77-92) 2. 76.9 3. 21.6 (95% CI 18.2-33.2)	1. 89 (95% CI 74-97) 2. 72.7 3. 19.7 (95% CI 16.4-26.5)	Placebo – mixture of six non-essential amino acids	1. DXA – Appendicular Skeletal Muscle Mass (kg) 2. At recruitment 3. Day 20	1. 14.1 (95% CI 10.9-21.6) 2. 14.0 (95% CI 11.6-24.9)	3. -0.1	1. Citrulline amino acid 2. 10g citrulline given once a day for 21 days	1. 11.8 (95% CI 9.9-20.4) 2. 13.3 (95% CI 11.4-18.5)	3. +1.5	NA	p=0.83 Favours null

		1. Deer 2. 2019 3. USA	1. General Medicine 2. Control n=20 3. Intervention n=20	1. 75.7 (7.1) 2. 70 3. 29.0 (5.3)	1. 80.0 (8.7) 2. 70 3. 28.9 (6.4)	Placebo isocaloric supplement, usual care	1. DXA FFM (kg) 2. During hospitalisation 3. Four weeks follow-up	1. 44.6 (9.8) 2. 45.1	3. +0.5 (0.9)	1. Whey protein 2. Twice daily 20g whey protein	1. 42.1 (8.9) 2. 42.2	3. +0.1 (1.6)	-0.4	p=0.72 *(all interventions vs. placebo)* Favours null
		1. Deer 2. 2019 3. USA	1. General Medicine 2. Control n=20 3. Intervention n=20	1. 75.7 (7.1) 2. 70 3. 29.0 (5.3)	1. 80.0 (8.8) 2. 70 3. 26.1 (6.6)	Placebo isocaloric supplement, usual care	1. DXA FFM (kg) 2. During hospitalisation 3. Four weeks follow-up	1. 44.6 (9.8) 2. 45.1	3. +0.5 (0.9)	1. Whey protein and rehabilitation 2. Chair-based exercises and resistance exercise and whey protein	1. 40.3 (9.7) 2. 40.9	3. +0.6 (1.6)	+0.1	p=0.72 *(all interventions vs. placebo)* Favours null
		1. Gade 2. 2019 3. Denmark	1. General medicine 2. Control n=82 Intervention n=83	1. 84.2 (6.3) 2. 65.3 3. 25.8 (5.2)	1. 85.3 (6.2) 2. 69.9 3. 25.1 (4.2)	Placebo isoenergetic beverage, resistance exercise, vitamin D	1. Bioelectrical Impedance Analysis – Lean Body Mass 2. Within 72 hours of admission 3. Within 72 hours of discharge	1. 42.5 (IQR 38.6 – 52.2) 2. 41.5	3. -0.1 (IQR -1.3 - +0.8)	1. Protein-enriched milk-based supplement 2. After breakfast and exercise	1. 44.0 (IQR 36.6 – 49.9) 2. 44.7	3. -0.3 (IQR -2.1 - +0.8)	NA	p=0.332 Favours null
Pharmaceutical	Physical Performance	1. Deer 2. 2019 3. USA	1. General Medicine 2. Control n=20 3. Intervention n=19	1. 75.7 (7.1) 2. 70 3. 29.0 (5.3)	1. 77.1 (7.4) 2. 74 3. 27.1 (5.3)	Placebo isocaloric supplement, usual care	1. SPPB 2. During hospitalisation 3. Four weeks follow-up	1. 7.8 (3.7) 2. 9.1	3. +1.3 (1.9)	1. Testosterone 2. Single IM dose	1. 7.4 (3.2) 2. 10.2	3. +2.8 (2.1)	+0.8	p=0.03 *(all interventions vs. placebo)* **Favours experimental**

	Muscle strength	1. Weissberger 2. 2003 3. UK	1. Elective orthopaedic 2. Control n=16 Intervention n=17	1. 67.3 (1.5) 2. 68.8 3. 27.1 (1.1)	1. 70.1 (1.6) 2. 70.6 3. 26.2 (1.2)	Placebo injection	1. Knee flexion 2. Pre-operatively at start of treatment 3. Four weeks post-operatively	1. Unknown 2. Unknown	3. -4.2%	1. Growth hormone 2. Once daily injections for 14 weeks preoperatively	1. Unknown 2. Unknown	3. -1.7%	NA	p=0.004 **Favours experimental**
		1. Hedström 2. 2004 3. Sweden	1. Orthopaedic surgery 2. Control n=9 Intervention n=11	1. 85 (3) 2. 75 3. 20.4 (1.8)	1. 83 (7) 2. 75 3. 22.8 (4.5)	Placebo	1.Knee extension (Nm) 2. Before treatment 3. End of treatment (21-28 days)	1. 10.5 (5.1) 2. 12.5	3. +2.0 (3.4)	1. Recombinant human growth hormone 2. Once daily subcutaneous injection for 21-28 days	1. 10.5 (4.1) 2. 12.9	3. +2.4 (3.1)	+0.1	p=0.8 Favours null
	Muscle mass	1. Hedström 2. 2004 3. Sweden	1. Orthopaedic surgery 2. Control n=9 Intervention n=11	1. 85 (3) 2. 75 3. 20.4 (1.8)	1. 83 (7) 2. 75 3. 22.8 (4.5)	Placebo	1. DXA – Lean Body Mass (kg) 2. Before treatment 3. End of treatment (21-28 days)	1. 38.1 (5.6) 2. 34.9	3. -3.2	1. Recombinant human growth hormone 2. Once daily subcutaneous injection for 21-28 days	1. 39.9 (6.2) 2. 39.2	3. -0.6	NA	p=0.03 **Favours experimental**
		1. Weissberger 2. 2003 3. UK	1. Elective orthopaedic 2. Control n=16 Intervention n=17	1. 67.3 (1.5) 2. 68.8 3. 27.1 (1.1)	1. 70.1 (1.6) 2. 70.6 3. 26.2 (1.2)	Placebo injection	1. Thigh cross sectional area 2. Pre-operatively at start of treatment 3. Four weeks post-operatively	1. Unknown 2. Unknown	3. -10.1%	1. Growth hormone 2. Once daily injections for 14 weeks preoperatively	1. Unknown 2. Unknown	3. +2.3%	NA	p=0.43 Favours null

		1. Deer 2. 2019 3. USA	1. General Medicine 2. Control n=20 3. Intervention n=19	1. 75.7 (7.1) 2. 70 3. 29.0 (5.3)	1. 77.1 (7.4) 2. 74 3. 27.1 (5.3)	Placebo isocaloric supplement, usual care	1. DXA FFM (kg) 2. During hospitalisation 3. Four weeks follow-up	1. 44.6 (9.8) 2. 45.1	3. +0.5 (0.9)	1. Testosterone 2. Single IM dose	1. 39.9 (6.4) 2. 39.6	3. -0.3 (1.4)	-0.9	p=0.72 *(all interventions vs. placebo)* Favours null
		1. Zhang 2. 2019 3. China	1. Orthopaedics 2. Control n=33 Intervention n=44	1. 78.6 (7.7) 2. 100 3. Unknown	1. 79.5 (6.2) 2. 100 3. Unknown	Usual care	1. DXA ASM (kg) 2. Before surgery 3. Four weeks post-operatively	1. 12.4 (1.2) 2. 12.5 (1.3)	3. +0.1	1. Erythropoietin injections 2. IM injections once daily for 10 days from day of surgery	1. 12.8 (1.5) 2. 13.0 (1.7)	3. +0.2	NA	p<0.001 **Favours experimental**
		1. Zhang 2. 2019 3. China	1. Orthopaedics 2. Control n=25 Intervention n=39	1. 75.0 (8.2) 2. 0 3. Unknown	1. 77.0 (7.7) 2. 0 3. Unknown	Usual care	1. DXA ASM (kg) 2. Before surgery 3. Four weeks post-operatively	1. 18.4 (1.8) 2. 18.4 (1.9)	3. +0.0	1. Erythropoietin injections 2. IM injections once daily for 10 days from day of surgery	1. 18.6 (1.7) 2. 18.8 (1.8)	3. +0.2	NA	p<0.001 **Favours experimental**
		1. Sloan 2. 1992 3. Canada	1. Orthopaedic surgery 2. Control n=14 Intervention n=15	1. 81 (6) 2. 100 3. Unknown	1. 83 (7) 2. 100 3. Unsknown	Placebo injections	1. Bioelectrical Impedance Analysis – Lean Body Weight (kg) 2. Within 48 hours of surgery 3. At four weeks or discharge	1. 35.6 (4.7) 2. 31.5 (4.8)	3. -4.1	1. Nandrolone decanoate injection 2. IM injection 2mg/kg weekly for four weeks	1. 31.6 (4.4) 2. 30.6 (3.2)	3. -1.0	NA	Not significantly different Favours null

NMES	Physical performance	1. Zinglersen 2. 2018 3. Denmark	1. Geriatric medicine 2. Control n=8 Intervention n=12	1. 84.9 2. 75 3. 26.1 (5.5)	1. 81.8 (8.9) 2. 75 3. 25.2 (4.1)	Functional training alone	1. Gait speed (m/s) 2. Within 2 days of admission 3. Day of discharge or maximum 10 days	1. 0.50 (0.20) 2. 0.61	3. +0.11	1. NMES 2. NMES combined with functional training (as detailed in physical activity section)	1. 0.50 (0.20) 2. 0.60	3. +0.10	NA	Not significant Favours null
		1. Lopez-Lopez 2. 2019 3. Spain	1. General medicine 2. Control n=47 Intervention n=48	1. 72.5 2. 42.6 3. 30.5 (5.7)	1. 74.9 2. 58.3 3. 25.9 (4.4)	Standard care	1. SPPB 2. Day of admission 3. At discharge	1. 4.2 (4.1) 2. 4.2 (3.2)	3. -0.0 (1.7) 4. p=0.563	1. NMES with rehabilitation 2. NMES with increasing levels of exercise	1. 3.6 (4.2) 2. 5.9 (3.6)	3. +2.3 (2.4) 4. p<0.001	+1.4	p=0.027 **Favours experimental**
	Muscle strength	1. Martin-Salvador 2. 2016 3. Spain	1. Respiratory 2. Control n=20 Intervention n=24	1. 77.4 (5.2) 2. 22 3. 28.9 (5.2)	1. 78.8 (6.3) 2. 16.8 3. 27.6 (3.8)	Usual care	1. Knee extension (kg) 2. Admission 3. Discharge	1. 10.5 (5.0) 2. 9.0 (3.4)	3. -1.5 (3.2) 4. p=0.005	1. Exercise and NMES combined 2. 30 minutes daily electric stimulation of both quadriceps	1. 11.1 (3.1) 2. 11.8 (4.2)	3. -0.7 4. p=0.408	+0.3	p=0.008 **Favours experimental**

8.7.3 Appendix 3 – Inclusion/ exclusion criteria for included studies

Author, date	Inclusion criteria	Exclusion criteria
Physical activity		
Busch, 2012	• ≥75 years • Coronary artery disease • Complete revascularisation after bypass graft • Able to start cardiac rehabilitation within 4 weeks after surgery • 6MWT 100 – 350m	• Exercise limiting comorbidities (e.g. orthopaedic or neurological) • Heart failure NYHA Class IV • Haemoglobin <90g/L • Wound healing disturbance • Cognitive or linguistic deficits • Peripheral artery occlusive disease
Blanc-Bisson[b] 2008	• >70years • Confined to bed or transferring from bed to chair with assistance • Independent locomotion within 3 months	• Neuromuscular diseases affecting lower limbs • Chronic respiratory failure • Heart failure NYHA Class IV • Peripheral vascular disease • Palliative care • Use of muscle-impairing drugs
Braun, 2019	• ≥65 years • Planned acute geriatrics stay ≥2weeks • Able to walk independently (with/without aid) or standby assistance • TUG > 9 sec	• Significant cognitive impairment • Severe hearing or visual impairment • Language barrier • Acute psychiatric problem • Palliative care • Any medical restriction on interventions
de Morton, 2007	• ≥65 years • Admitted to either of two medical wards with a general medical condition	• Admitted from nursing home • Assessed to need nursing home level or palliative care

		• Stroke or condition for which mobilisations contraindicated (e.g. fracture) • Too unwell to ambulate or exercise • Readmitted following previous participation in study
Deer, 2019	• ≥65 years • Residing at home before/ after admission • Self-reported ability to walk across small room two weeks before admission • Able to stand independently at baseline testing	• Uncontrolled hypertension • History of stroke with motor disability • Renal or liver insufficiency • Anabolic steroids within 3 months • Planned hospitalisation within 30 days of discharge • Cognitive impairment • Living more than 30miles from hospital
Fiore[b], 2017	• >18years • Planned colorectal resection	• Known metastases • Neurological or musculoskeletal conditions that preclude postoperative mobilisation • Unable to speak English or French • Critical care admission straight after surgery
Giangregorio, 2009	• Treated surgically for hip fracture • Stable fracture or adequate fixation • Able to follow two step commands • Able to take few steps with help of assistive device	• In isolation • Cultures positive for MRSA • Able to walk without assistive devices • Hip, knee, or ankle surgery before hip fracture • Unable to give informed consent • Incontinent • Uncontrolled cardiovascular disease, Diabetes Mellitus, or hypertension

		• Neuromuscular disease or other musculoskeletal disease
Henriksen[b], 2002	• Referred for elective colorectal surgery	• Inflammatory bowel disease • Disseminated cancer • Serious cardiopulmonary disease
Houborg, 2006	• ≥60 years • Referred for elective colorectal surgery	• Living more than 40km from hospital • Inflammatory bowel disease • Disseminated cancer • Significant psychiatric disease or dementia • Other medical reasons that precluded physical training
Jones, 2006	• ≥65 years • General medical admission • Able to give informed consent	• Nursing home resident or nursing level of care at home • Medically unstable • Mobilisation contraindicated by treating medical team • Admitted to delirium management unit • Non weight-bearing • Requiring palliative care • Diagnosis known to cause functional impairment (e.g. stroke, fracture) • Expected LoS <24 hours
Martinez-Velilla, 2019	• ≥75 years • Barthel index ≥60 • Able to ambulate with/ without assistance • Able to communicate and collaborate with research team	• Expected LoS <6 days • Very severe cognitive decline • Terminal illness • Uncontrolled arrhythmias • Acute pulmonary embolism • Recent myocardial infarction • Recent major surgery

		• Extremity bone fracture in the past 3 months
McCullagh, 2017	• ≥65 years • General medical admission • Anticipated LoS >3days • Needed either an aid or assistance to walk on admission	• Medically too unwell • Contra-indication to exercise present e.g. hip fracture, uncontrolled heart rate • Assistance of more than one person to walk safely required • Baseline SPPB 0 or 1 • Admitted for surgical, critical, end of life, or psychiatric care • Unable to follow commands in English (language or too confused/ agitated) • Participated in trial within previous 12 months
McGowan [b], 2018	• ≥65 years • Admitted to hospital within preceding 48 hr • Able to sit in a chair independently • Able to follow 1-stage command	• Predicted discharge within next 48hr • Terminally ill or moribund • Needing isolation precautions • Bedbound prior to admission • Condition that made them unable to use pedal exerciser
Moseley, 2009	• Surgical fixation for hip fracture admitted to rehabilitation wards • Approval to weightbear or partial weightbear • Able to tolerate exercise programmes • Able to take ≥4 steps with forearm support frame and assistance of one person • No medical contraindications limiting ability to exercise	• >4 adjusted errors on Short Portable Mental Status Questionnaire and no carer available to supervise exercise programme • Discharged directly from acute orthopaedic ward

	• Living at home or low care residential facility prior to fracture with plan to return on discharge	
Ortiz-Alonso, 2019	• >75 years-old • Admitted to acute care of elderly unit during recruitment dates	• Nonambulatory or dependent in all ADLs 2weeks before admission • Unstable cardiovascular disease or other major condition contraindicating exercise • Terminal illness • Dementia • LoS <3days • Death prior to discharge • Scheduled admission • Transferred from another hospital
Opasich, 2010	• >70 years • Medically stable patients (i.e. without acute diseases such as acute heart failure, systemic infection, acute respiratory failure etc.) • Admitted to unit after cardiac surgery	• Mini Mental State Examination score < 20
Prasciene, 2019	• ≥65 years • Valve surgery or intervention • Ability to start rehabilitation within 4weeks of surgery • 6MWT 100-350m • Written consent	Not specified
Rahmann, 2009	• Planned primary hip or knee replacement for osteoarthritis • Home visit not possible prior to admission	• Diagnosed neurologic disorder • Another major musculoskeletal disorder that altered mobility

		• Cognitive dysfunction • Undergoing revision joint surgery or bilateral knee replacements • Specifically requested aquatic physiotherapy postoperatively and not willing to be randomised
Raymond, 2017	• ≥65 years • Admitted to geriatric medicine ward • Able to participate in weight-bearing exercise i.e. adequate exercise tolerance, able to stand from chair with minimum/no assistance	• Medical instability • Pre-morbidly non-ambulant • Mini Mental State Examination score <10 • Admitted for palliation • Weight-bearing restrictions • Planned discharge < 7 days • Inappropriate behaviour or cognition for group exercises
Said [b], 2012	• ≥60 years • Improve mobility/ walking listed as goal on admission	• Primary reason for admission was to await residential care placement • Did not require physiotherapy • Medical restrictions on mobilisation • Non-English speaking and advocate not available
Said [b], 2018	• Admitted to four participating geriatric rehab wards at two hospitals • Aged > 60 • Goal to "improve mobility or walking" determined by admission referral or treating therapist • Informed consent was obtained from the participant or 'responsible	• Medical restrictions limiting mobilisation • Goals were non-weightbearing • Enrolled in another randomised trial • Primary reason for admission was carer training or residential care placement

	person' within 48 hours of admission, with interpreters utilised as necessary	
Sano, 2018	• >60 years • Able to walk >10m without assistance one week after total knee arthroplasty	• Any medical or neurological problem affecting ability to complete trial e.g. stroke, cardiac insufficiency, acute respiratory failure
Schwenk, 2014	• Dementia confirmed • Written informed consent or informed legal guardian • Age > 65 • No delirium • No aphasia • No severe visual or auditory impairment • No severe psychiatric disorders • No contraindications for intensive resistance and functional training such as orthopaedic instability, hernia, or uncontrolled disorders	• No additional exclusion criteria specified
Sherrington, 2003	• Admitted to rehabilitation ward following recent fall-related hip fracture	• <60 years • Unable to complete assessments and exercise program due to one or more of a) cognitive impairment b) major medical conditions c) complications from fracture directed to be non-weightbearing
Tal-Akabi[b,] 2007	• Admitted to musculoskeletal rehabilitation unit after lower limb surgery	• Neuromuscular, cardiovascular, or other disorders that could influence and/or limit participation in tailored strength training programme • Taking corticosteroids or anabolic drugs

Torres-Sánchez, 2017	• ≥65 years • Admitted to respiratory ward due to acute exacerbation of Chronic Obstructive Pulmonary Disease	• Inability to provide informed consent • Presence of psychiatric or cognitive disorders • Severe orthopaedic problems • Organ failure • Cancer • Inability to cooperate • Another exacerbation in previous month • Did not complete at least four days of intervention
Wnuk, 2016	• Males aged 65-75 years • Stable cardiovascular disease • Absence of neurological disorders • Non-symptomatic aneurysm	• Neurological disorders • Unstable coronary heart disease • Symptomatic aortic aneurysm • Aortic dissection • Having difficulty in locomotion • Not able to start physical training first or second day after surgery • Psychiatric diseases • Lack of compliance with physiotherapist • Other medical contraindications
Nutrition		
Beelen, 2017	• ≥65 years • Admitted to general, geriatric, or respiratory medicine	• Hospital stay expected to be <4 days • Terminally ill • Food allergy or intolerance that restricted them from receiving standard energy and protein-rich menu/ protein-enriched intervention products • eGFR ≤30

		• Communication difficulties - aphasia or not understanding Dutch • Delirium diagnosis • At risk for developing refeeding syndrome
Bouillanne, 2018	• >70 years • Moderate undernutrition	• Severe cognitive impairment • Severe inflammation (CRP>50) • Diabetes mellitus • Being fed by parenteral or enteral nutrition • Severe renal insufficiency • Class IV heart failure • Severe liver disease • Documented intestinal insufficiency • Respiratory failure • Chronic infectious or inflammatory disease • Corticosteroid medications or progressive cancer
Deer, 2019	• ≥65 years • Residing at home before/ after admission • Self-reported ability to walk across small room two weeks before admission • Able to stand independently at baseline testing	• Uncontrolled hypertension • History of stroke with motor disability • Renal or liver insufficiency • Anabolic steroids within 3 months • Planned hospitalisation within 30 days of discharge • Cognitive impairment • Living more than 30miles from hospital
Ekinci, 2016	• Female hip fracture patients • ≥65 years	• Diabetes Mellitus • Organ failure

	• Ambulatory pre-fracture • Nutritional risk screening 2002 ≥3 and followed by hospital nutrition support team	• Renal and hepatic failure • Gastrointestinal intolerance • Endocrine pathology e.g. thyroid disorders • Dementia
Files, 2020	• ≥55 years • PaO_2:FiO_2 <300 • Mechanical or non-invasive ventilation • Resolving respiratory failure	• Mechanical ventilation for > 7days • Current hospitalisation > 14 days • Inability to walk previously (with or without aid) • Injury causing inability to walk or perform functional tests • Neuromuscular disease • Pregnancy - Non-verbal prior to acute illness • Acute stroke • BMI > 50 • Body weight <= 60kg • Cancer treatment within the last 6 months • Moribund • Participation in another research study • Current use of nitroglycerine or nitrate preparations • Current use of PDE type 5 inhibitors • Inability to take drug by oral or nasogastric tube • Active gastrointestinal bleeding • Renal replacement therapy • Severe liver disease
Gade, 2019	• ≥70 years	• Active cancer or terminal disease

	• Danish speaking • Expected LoS >3days • Independent stand function >30sec	• Renal insufficiency • Cognitive impairment • Parenteral nutrition only • Milk or lactose allergy or intolerance • Weight loss plan or special diet • Permanent Pacemaker
Hermanky, 2017	• ≥65 years • Surgically treated hip fracture	• Pathological fracture • Severe renal insufficiency or the need for dialysis • Presence of a severe cognitive impairment • Implanted Pacemaker • Refusal of the consumption of animal food
Niccoli, 2017	• ≥60 years • Ability to perform functional tests (with or without use of an assistive device) • Willing to give informed consent and be randomised	• NYHA Class III or IV heart failure • Clinically significant aortic stenosis, history of cardiac arrest, cardiac defibrillator, or uncontrolled angina • Lung disease requiring oral or injected steroids or use of supplemental oxygen • Modified mini-mental state <70 • Severe arthritis • Cancer requiring treatment in past 3 years • Parkinson's or other serious neurological disorders • Renal disease requiring dialysis • Other illness of such severity that life expectancy considered to be less than 12 months

		• Current diagnosis of schizophrenia, other psychotic disorders, or bipolar disorder • Current consumption of more than 14 alcoholic units/ week • Clinical judgement concerning participant safety or noncompliance
Ogasawara, 2018	• Diagnosed Chronic Obstructive Pulmonary Disease • Hospitalised for acute exacerbation or community-acquired pneumonia • Planned to receive pulmonary rehabilitation • Able to eat and drink safely	• History of severe drug allergy • Taking oral nutritional supplements during the trial • Uncontrolled diabetes mellitus or dyslipidaemia • Refused pulmonary rehabilitation
Pedersen, 2019	• ≥65 years • Admitted with acute illness from own home to emergency department	• Terminal illness or cancer treatment • COPD • Living outside three identified municipalities • Unable to speak Danish • Inability to cooperate with tests/ exercises • Critical care admission • Expected LoS <2days • Inability to stand
Saudny-Unterberger, 1997	• Consecutive patients aged 40 to 85 • Admitted to chest institute • Diagnosis of Chronic Obstructive Pulmonary Disease • FEV1 ≤60% predicted • Able to give informed signed consent	• Required mechanical ventilation • Gastrointestinal tract disorder • Active cancer or other condition predisposing to weight loss • Terminally ill • Unable to communicate in English or French • Mental confusion

		• Followed a special diet
Pharmaceutical		
Deer, 2019	• ≥65 years • Residing at home before/ after admission • Self-reported ability to walk across small room two weeks before admission • Able to stand independently at baseline testing	• Uncontrolled hypertension • History of stroke with motor disability • Renal or liver insufficiency • Anabolic steroids within 3 months • Planned hospitalisation within 30 days of discharge • Cognitive impairment • Living more than 30miles from hospital • For testosterone arm: history of breast/prostate cancer, palpable prostate nodule, raised PSA, low haematocrit, decompensated heart failure
Hedström, 2004	• >65 years • Previously ambulant, not cognitively impaired • Femoral neck or trochanteric fracture	• Treated with GH during the last 12 months • Severe illness during the last 6 months • Major surgery within 1 month • Glaucoma • Insulin-treated diabetes mellitus • Current or previous malignant disease • Severe liver or renal disease • Known or suspected alcohol abuse • Suspected to be non-cooperative.
Sloan, 1992	• Elderly patients with hip fractures admitted to orthopaedic surgery	• In extended care prior to admission • <65 years • Severe dementia

		• Severe medical illnesses e.g. congestive heart failure, metastatic cancer • Hormone responsive tumours • Prostatic obstruction • Liver disease
Weissberger, 2003	• Awaiting elective total hip replacement for osteoarthritis • In good general health • Without evidence of significant renal impairment, liver disease, diabetes mellitus, poorly controlled hypertension, or malignancy (past or current)	• Not meeting inclusion criteria
Zhang, 2019	• ≥60 years • Femoral intertrochanteric fracture • Able to sign written consent form • First hip surgery • Anaemia due to surgical perioperative red blood cell mobilisation	• Diabetes Mellitus • Ongoing cancer treatment • Nerve or muscle dysfunction • Other diseases caused by limited physical activity • Serious underlying diseases
Neuromuscular Electrical Stimulation		
Lopez-Lopez, 2019	• Elderly patients hospitalised due to pneumonia (community-acquired)	• Hospital-acquired pneumonia • Musculoskeletal or neurological conditions that might interfere with the evaluation or intervention • Intervention group patients exhibiting changes in mental status • Likely to leave hospital within 5 days • Inability to complete any of the interventions

Martin-Salvador, 2016	• Aged 65-90 • FVC <60% • Admitted for community-acquired pneumonia or acute exacerbation of COPD	• Significant cognitive impairment • Effusion, pneumothorax or haemoptysis • Cancer • Dermatological or venous insufficiency, with osteo-synthesis material • Could not perform the evaluation • In isolation • Admitted within previous two weeks
Zinglersen, 2018	• ≥65 years • Admitted to geriatric medicine ward	• Cognitive impairment • Inability to give informed consent • Dementia • Severe memory impairment • Delirium • Non-Danish speaking • More than one assistant to enable mobilisation • Unable to rise from chair without prominent armrest support or >9 repetitions in 30s chair stand test • Terminal cancer, severe COPD, severe heart failure, isolated due to infectious disease • Expected LOS < 6 days

8.7.4 Appendix 4 – Full risk of bias results for included studies

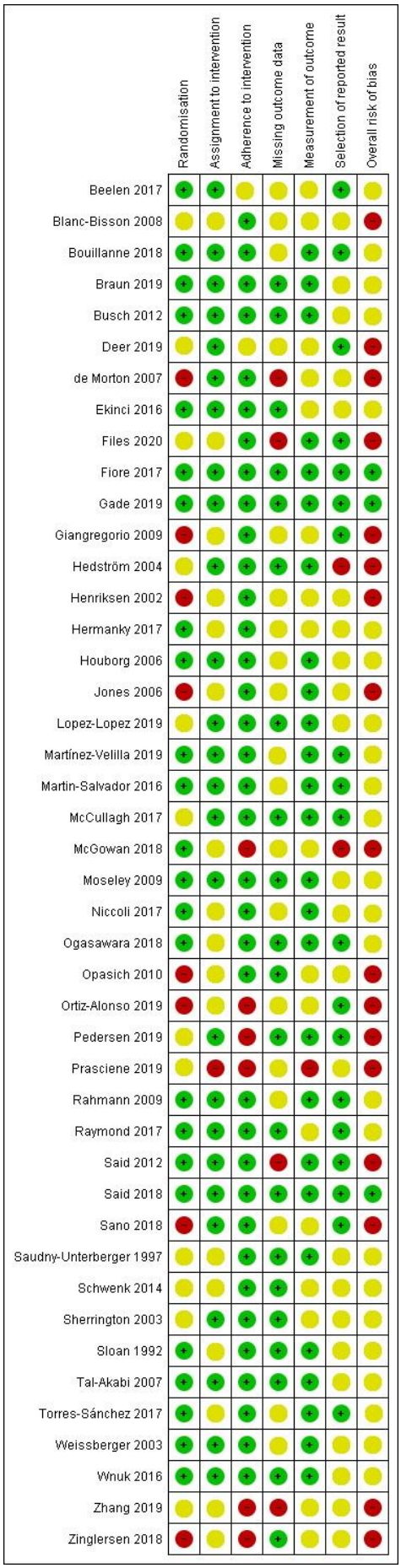

Figure 8.7-1 – Risk of bias results for each individual included study.

Green circles denote low risk of bias, yellow circles denote some concerns, and red circles denote high risk of bias.