Skeletal Muscle Mass in Critically ill Adults:

Assessment, Changes, and Association with Nutrition Delivery

Submitted by

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Table of Contents

Abstract

Low muscle mass at intensive care unit (ICU) admission and increased muscle wasting during critical illness negatively influence patient recovery. The reasons for muscle wasting are complex and inadequate nutrition delivery may play a role. Progressing the characterisation of muscle wasting is complicated by the lack of easily accessible and routinely used reference methods for assessing muscle mass in ICU patients. This thesis sought to evaluate the utility of easily applied bedside methods to measure muscularity and investigate the association of energy and protein delivery and changes in muscle health indices during critical illness. Details of the research studies are below:

1. A prospective observational study comparing muscularity assessed by bedside methods (a multi-site ultrasound protocol, bioimpedance spectroscopy [BIS], arm anthropometry, and subjective physical assessment) with a reference method (computed tomography [CT] image analysis at the third lumbar area) in 50 patients at ICU admission. The ultrasound protocol including the mid-upper arm and bilateral thighs and a BIS-derived fluid-adjusted fat-free mass variable had strong correlations with CT-measured muscularity and correctly identified patients with low CT muscle area.
2. A systematic literature review investigating associations between energy and protein delivery and skeletal muscle mass changes in critical illness finding that there is insufficient good quality evidence to substantiate a relationship between the two.
3. A retrospective study investigating changes in CT-measured muscle mass and quality in 32 critically ill patients observed marked muscle wasting over the first month of ICU admission, attenuated after weeks 5-7. Energy and protein delivery were not associated with the degree of muscle loss.

These studies will inform future development of ultrasound and BIS as methods for muscle assessment in the ICU setting and assist in designing studies investigating the impact of nutrition therapy on skeletal muscle mass changes in acute illness.

Statement of Authorship

This thesis includes work by the author that has been published. Except where reference is made in the text of the thesis, this thesis contains no other material published elsewhere or extracted in whole or in part from a thesis accepted for the award of any other degree or diploma.

No other person's work has been used without due acknowledgment in the main text of the thesis.

This thesis has not been submitted for the award of any degree or diploma in any other tertiary institution.

All substantive contributions by others to the work presented including jointly authored publications, is clearly acknowledged.

All research procedures reported in the thesis were approved by the relevant Ethics Committee (Appendix 1).

Katherine Lambell

Date: 27th July, 2021

Candidates Declaration

For Chapters 3 to 6, the extent of the candidate's contribution was as follows:

Chapter	Publication Title	Publication Status	Contribution
3	Comparison of ultrasound-derived muscle thickness with computed tomography muscle cross-sectional area on admission to the intensive care unit: a pilot cross-sectional study	Published	70%
4	How does muscularity assessed by bedside methods compare to computed tomography muscle area at intensive care unit admission? A pilot prospective cross-sectional study	Published	80%
5	Association of energy and protein delivery on skeletal muscle mass changes in critically ill adults: a systematic review	Published	85%
6	Marked losses of computed tomography–derived skeletal muscle area and density over the first month of a critical illness are not associated with energy and protein delivery	Published	80%

Signed: Date: 17th July, 2021

Supervisors Declaration

I hereby certify that the declaration above is a correct reflection of the extent of the contributions made by the student candidate towards each chapter in the thesis.

Name of Supervisor
Susannah King

The extent and nature of contributions of the student candidate and all co-authors towards each study have been clearly acknowledged at the beginning of Chapters 3 to 6.

List of Figures

List of Tables

List of Abbreviations

APACHE	Acute Physiology and Chronic Health Evaluation
AUC	Area Under the Curve
BIA	Bioimpedance Analysis
BIS	Bioimpedance Spectroscopy
BMI	Body Mass Index
CSA	Cross-sectional Area
CT	Computed Tomography
ECW	Extracellular Water
FFM	Fat-free Mass
GLIM	Global Leadership Initiative on Malnutrition
ICU	Intensive Care Unit
ICW	Intracellular Water
MAC	Mid-upper Arm Circumference
MAMC	Mid Arm Muscle Circumference
RCT	Randomised Controlled Trial
ROC	Receiver Operating Characteristic
SGA	Subjective Global Assessment
TBW	Total Body Water

Publications and Presentations

Publications directly arising from this thesis:

The following published manuscripts were conducted and written during the period of candidature for the specific purpose of obtaining the degree of Doctor of Philosophy. Each manuscript was formatted in accordance with the requirements specific to the relevant journal. Sections of this thesis that were not submitted for publication use the *JAMA* referencing style, modified to include all authors in the reference section, and are written in Australian English.

1. **Lambell KJ**, Tierney AC, Wang JC, Nanjayya V, Forsyth A, Goh GS, Vicendese D, Ridley EJ, Parry SM, Mourtzakis M, King SJ. Comparison of ultrasound-derived muscle thickness with computed tomography muscle cross-sectional area on admission to the intensive care unit: a pilot cross-sectional study. *JPEN*. 2021 Jan;45(1):136-45. DOI: 10.1002/jpen.1822
2. **Lambell KJ**, Earthman CP, Tierney AC, Goh GS, Forsyth A, King SJ. How does muscularity assessed by bedside methods compare to computed tomography muscle area at intensive care unit admission? A pilot prospective cross-sectional study. *J Hum Nutr Diet*. 2021 Apr;34(2):345-55. DOI: 10.111/jhn.12804
3. **Lambell KJ**, King SJ, Forsyth AK, Tierney AC. Association of energy and protein delivery on skeletal muscle mass changes in critically ill adults: a systematic review. *JPEN*. 2018 Sep;42(7):1112-22. DOI: 10.1002/jpen.1151
4. **Lambell KJ**, Goh GS, Tierney AC, Forsyth A, Nanjayya V, Nyulasi I, King SJ. Marked losses of computed tomography–derived skeletal muscle area and density over the first month of a critical illness are not associated with energy and protein delivery. *Nutrition*. 2021. DOI: 10.1016/j.nut.2020.111061

Published abstracts arising from this thesis:

1. ASPEN NUTRITION SCIENCE & PRACTICE CONFERENCE: March 28-31, 2020, Tampa, Florida: Vars Candidates, Trainee Awards, Best of ASPEN (Topic Awards), International Awards, Abstracts of Distinction, Posters of Distinction and Other Abstracts. *JPEN*. 2020;44(2):382. Supplement 29, page 102

Other publications during candidature:

1. Chapple L, Tatucu-Babet O, **Lambell KJ**, Fetterplace K, Ridley EJ. Nutrition guidelines for critically ill adults admitted with COVID-19: Is there consensus? *Clin Nutr ESPEN*. 2021. DOI: 10.1016/j.clnesp.2021.05.003
2. **Lambell KJ**, Miller EG, Tatucu-Babet OA, Peake S, Ridley EJ. Nutrition management of obese critically ill adults: A survey of critical care dietitians in Australia and New Zealand. *Aust Crit Care*. 2021 Jan 1;34(1):3-8.
3. **Lambell KJ**, Tatucu-Babet OA, Chapple LA, Gantner D, Ridley EJ. Nutrition therapy in critical illness: a review of the literature for clinicians. *Crit Care*. 2020 Dec 1;24(1):35
4. **Lambell KJ**, Tatucu-Babet OA, Ridley EJ. Response to the letter to the editor: "Using indirect calorimetry in place of fixed energy prescription was feasible and energy targets were more closely met: do not forget an important limitation". *Crit Care*. 2020 Dec;24(1):1-2
5. Tatucu-Babet OA, Fetterplace K, **Lambell K**, Miller E, Deane AM, Ridley EJ. Is energy delivery guided by indirect calorimetry associated with improved clinical outcomes in critically ill patients? A systematic review and meta-analysis. *Nutr Metab Insights*. 2020. DOI: 10.1177/1178638820903295

6. **Lambell K**, Peake S, Ridley E. Nutrition management of obese critically ill patients: More research is urgently needed. *Clin Nutr*. 2019 Aug 1;38(4):1957.
7. **Lambell K**, King S, Ridley E. Identification of malnutrition in critically ill patients via the subjective global assessment tool: more consideration needed? *J Intensive Care Med*. 2017 Jan;32(1):95.

Accepted oral abstract presentations during candidature:

1. Australasian Society for Parenteral and Enteral Nutrition Annual Scientific Meeting, Adelaide, "Can bedside ultrasound be used to measure muscularity at ICU admission?" November 2019
2. Australasian Society for Parenteral and Enteral Nutrition Annual Scientific Meeting, Adelaide "Nutrition management of critically ill obese patients in Australia and New Zealand" November 2019
3. Australian and New Zealand Intensive Care Society Clinical Trials Group meeting, novice investigator session, Noosa "Evaluation of bedside methods to measure muscularity at the bedside in critical illness" March 2019
4. Australasian Society for Parenteral and Enteral Nutrition Annual Scientific Meeting, Melbourne "Association of energy and protein delivery on skeletal muscle changes in critical illness" November 2016

Grants and Awards

Grants

Grants relating to research in this thesis:

Grant	Value
Travel scholarship to attend and present at the American Society for Parenteral and Enteral Nutrition Science and Practice conference, Tampa, Florida; La Trobe University, 2020 (due to the COVID-19 pandemic the travel was cancelled)	$2,500
Travel scholarship to travel to Canada and America to build collaborations with key body composition researchers and train in body composition assessment (CT and ultrasound); La Trobe University, 2016	$1,500
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Grant	Value
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Dedication

Oscar & Rosie

“Ambition is the path to success. Persistence is the vehicle you arrive in.”

-Bill Bradley

Chapter 1

Introduction

1.1 Context of thesis

Patients admitted to an intensive care unit (ICU) with life-threatening illnesses often require specialist medical management and organ support. More than 160,000 patients are admitted to ICUs in Australia each year, incurring billions of dollars in healthcare costs[1]. Over time, advances in medical treatments have resulted in more critically ill patients surviving an ICU admission (approximately 90% in Australia)[2]. However, increased survival rates are associated with higher rates of long-term morbidity following critical illness, with data suggesting new and continued disability in physical function, cognition, and health-related quality of life up to five years after ICU admission[3-5]. Improving the *quality of survival* has been arguably described as *"the defining challenge of critical care in the 21st century"*[6].

Skeletal muscle is the most abundant tissue in the human body. Baseline muscularity has been identified as an important predictor of recovery from critical illness[7-9]. The rapid and significant muscle wasting which occurs in acute critical illness is belived to be a major contributor to long-term disability after ICU admission[10-13]. Therefore, attenuation of muscle loss may assist with optimising quality of survival. The cause and mechanisms for these marked losses in skeletal muscle mass are multifactorial and nutrition delivery and adequacy may play a role[14]. A focus on technology that can be easily applied at the bedside is required to: 1) identify patients who have low muscle mass who may benefit from early intervention and 2) evaluate interventions aimed at attenuating deterioration in muscle mass and quality in critical illness.

This thesis focuses on the application and evaluation of bedside body composition technology to assess muscularity at ICU admission; and investigates the association between energy and protein delivery and changes in skeletal muscle mass and muscle quality during critical illness. Chapter 1 provides a broad overview of critical illness (section 1.2), followed by a discussion of the clinical importance of skeletal muscle in critical illness (section 1.3), a summary of body composition terminology and methods (section 1.4), an overview of nutrition assessment and therapy in critical illness (section 1.5), a summary which highlights gaps in the literature (section 1.6), and concludes with the overall thesis aims and outline (section 1.7).

1.2 Overview of critical illness and the intensive care setting

The term *critical illness* refers to a range of clinical conditions and situations where patients are predominately cared for in an intensive care unit[15]. Although critical illness is frequently characterised by organ failure and/or sepsis, patients may be admitted to an ICU with a range of other life-threatening conditions such traumatic injuries, burns, haemorrhage, ischaemia, and following major surgery for monitoring and/or organ support. Critically ill patients are a heterogeneous group, with differing admission diagnoses and pre-morbid status. Some patients will be admitted to an ICU for a few days for monitoring (e.g. post cardiac surgery), while others may be critically ill for weeks and months, and require ongoing specialist procedures and life-saving organ support (e.g. mechanical ventilation, continuous renal replacement therapy, extracorporeal membrane oxygenation).

With regard to terminology, within the clinical and academic critical care fields, various terms such as ICU care, critical care, intensive care, are used interchangeably. Additionally, the acronym for "Intensive Care Unit" is commonly written as ICU or ITU. In this thesis the terms critical care and ICU are used.

1.2.1 Critical care in Australia

Registry data from the 2018/2019 Australian and New Zealand Intensive Care Society Critical Care Resources Survey reported there were 220 ICUs across Australia and New Zealand at that time[16]. This includes a mix of adult and paediatric metropolitan and rural/regional ICUs.

Within each ICU there is a differing number of beds allocated to patients requiring intensive care or high dependency care. Usually, patients who require intensive care need one-to-one nursing care, while high dependency patients are cared for by a ratio of one nurse to two patients (e.g. post-surgical monitoring). The level of care required depends on many factors including the organ support therapies they are receiving. The admitting source and acuity of patients within each ICU differs depending on the case-mix of the hospital they are servicing and the type of ICU (private versus public) and location (metropolitan versus regional). For example, private ICUs in Australia have a higher proportion of post-surgical admissions than public hospitals (75% versus 25%, respectively) and metropolitan tertiary hospitals will typically run state-wide clinical services (e.g., trauma, burns, organ transplant, extracorporeal membrane oxygenation)[16].

The critical care multidisciplinary teams commonly include a team of specialist intensive care medical doctors, nurses, pharmacists, dietitians, physiotherapists, social workers, and speech pathologists. Models of care may vary from hospital to hospital and each hospital will have its own governance arrangements for which medical team has primary responsibility for a patient admitted to ICU. Typically, the ICU team will work in close consultation with the primary medical, trauma, or surgical team.

Interventions provided to critically ill patients are wide ranging, and include life-saving organ and therapeutic support, along with physical and nutrition therapy. In particular, the early provision of enteral nutrition (within 48 hours of ICU admission) in patients who are mechanical ventilated is an established standard of care and supported by international clinical guidelines[17,18]. The key nutrition assessment methods and interventions provided to critically ill patients are outlined in section 1.5.

1.2.2 Pathophysiology of critical illness

1.2.2.1 Phases of critical illness

When a life-threatening condition triggers a "critical illness", survival is influenced by the ability of the body to mount an appropriate adaptive response[19]. This response involves complex metabolic, hormonal, and immunological changes, and multiple organ systems[19]. Specifically, in 1942, Sir David Cuthbertson described two distinct metabolic phases during acute illness—the 'ebb' (early shock) phase, followed by the 'flow' (catabolic) phase[20]. The 'ebb' phase is characterised by haemodynamic instability and hormonal changes (including insulin resistance) in order to prioritise the delivery of energy substrates to vital tissues[20,21]. This survival mechanism results in endogenous glucose production as well as

transiently lower energy expenditure compared to pre-injury or illness[22]. This physiological response to critical illness is vital for immediate survival. The 'flow' phase is characterised by the breakdown of tissue (including skeletal muscle) in order to provide substrates to cover the immediate needs for the 'fight or flight' response (e.g. muscle broken down to provide amino acids use for gluconeogenesis), while energy expenditure increases[22].

More recently, a third, anabolic recovery phase has been described[22,23]. It is during this phase when energy expenditure remains high, resynthesis of lost tissue is thought to take place and the body may be more metabolically able to process delivered nutrients[22,23]. Currently there are no clinical biomarkers available to identify when patients move through the different metabolic phases, and the duration of each phase will be dependent on the type and severity of illness, effectiveness of therapies, and complications that may be experienced[22]. However, to enable practical recommendations for the delivery of nutrition while taking into account the metabolic changes, the European Society for Parenteral and Enteral Nutrition (ESPEN) clinical guidelines have suggested the early 'ebb' phase of decreased metabolism typically occurs around ICU day 1–2, the 'flow' catabolic phase from ICU day 3–7, and the anabolic recovery phase after ICU day 7, Figure 1.1[18].

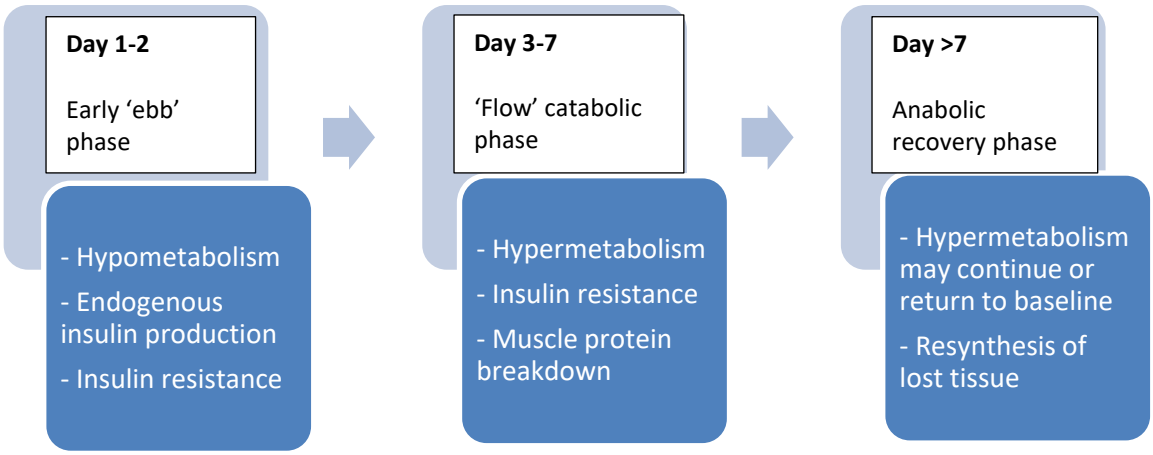


Figure 1.1 Phases of critical illness and key metabolic consequences, adapted from ESPEN guidelines[18]

1.2.2.2 Influences on metabolic rate in critical illness

In addition to the physiological response outlined above, multiple other factors can influence metabolic rate in critical illness. For example, metabolic rate can be decreased by hypothermia, muscle relaxants, and analgesia[24]. Increased metabolic rate can be induced by fever, shivering, work of breathing, and injury severity (with the degree of hypermetabolism proportional to the degree of injury/illness)[24].

1.2.2.3 Protein metabolism in critical illness

Skeletal muscle mass is maintained through regulation of muscle protein synthesis and protein breakdown and is influenced by different anabolic and catabolic stimuli[25]. In critical illness, depressed muscle protein synthesis relative to muscle protein breakdown, leads to a net catabolic state and rapid and significant skeletal muscle wasting, with the amount of muscle loss being relative to the severity of critical illness[11,26]. Over time, this

net catabolic state is believed to be reduced mostly due to increased muscle protein synthesis over time (from around ICU day 30)[27]. Section 1.3.2 discusses the changes in skeletal muscle mass and quality reported in the critical care literature.

The mechanisms for altered protein homeostasis in critical illness are still largely unknown but are believed to be multifactorial and include components as detailed in Table 1.1 and 1.2 below.

Table 1.1 Reasons for increased muscle protein *breakdown* in critical illness

Reason	**Details**
Activation of the ubiquitin proteasome system (UPS)	• The UPS mediates muscle protein breakdown in the critically ill[11,28] • A number of stimuli common in critical illness can activate the UPS (e.g., inflammation, oxidative and energy stress, abnormal lipid metabolism, and immobilisation)[28]
Dysregulation of autophagy	• Autophagy is the natural, regulated mechanism of the cell that removes unnecessary or dysfunctional components[29] • Dysregulation of autophagy has been associated with muscle wasting in critical illness. Specifically, impairment in autophagy promotes muscle loss due to the accumulation of damaged and toxic proteins within the muscle[29-31]
Altered substrate ultilisation	• Carbohydrate metabolism: hyperglycemia and insulin resistance are common in early critical illness (further details are provided in Table 1.2). The release of catabolic hormones stimulated by pro-inflammatory cytokines, results in glycogenolysis and gluconeogenesis in the liver to mobilise glucose for utilisation by tissues and cells that require glucose as their primary energy source (e.g. central nervous system and inflammatory cells)[15]. Glycogen stores are quickly depleted and endogenous fat and protein become the major source of energy[15]. With the absence of an exogenous source of glucose there is significant protein degradation (i.e. muscle breakdown to supply amino acids as substrate for gluconeogenesis)[15]

Table 1.2 Reasons for decreased muscle protein *synthesis* in critical illness

Reason	**Details**
Decreased mitochondrial number and/or function	• Muscle protein synthesis is ATP dependent[32] • In acute critical illness, reduced muscle protein synthesis may be the result of decreased mitochondrial number and/or function, which may be the result of decreased substrate utilisation including glucose[32]
Altered substrate metabolism	In health, skeletal muscle is highly responsive to nutrition interventions. However, in critical illness, recent evidence has indicated that that there is a decreased ability for the body to utilise substrates for muscle protein synthesis as discussed below[26]: • Amino acids: the availability of amino acids is an important prerequisite for muscle maintenance as they are the precursors required to synthesise muscle protein. Furthermore, at the cellular level, an intracellular abundance of amino acids activates the mammalian target of rapamycin (mTOR), which is the main pathway for driving muscle protein synthesis[33]. In health, there are reports of 11% of diet-derived protein being directly incorporated into muscle myofibrillar protein[34]. However, in critical illness it is believed that delivered protein is diverted away from the muscle and amino acids released into the circulation to meet the increased metabolic demands of the body[19,26] • Fatty acids: in acute critical illness, carbohydrate is the preferred energy substrate over fats. Conversion of fat to ATP requires large amounts of oxygen and fully functioning mitochondria, both which are impaired in critical illness[15]. Stress hormones stimulate lipase. In critical illness, the overall metabolism of fats is increased, but complete oxidation can only happen in tissues where there are functioning mitochondria[15]. A recent study conducted in 63 critically ill patients, indicated that in early critical illness neither muscle ATP content nor muscle mass was related to the quantity of fatty acids delivered as part of nutrition or sedative use (i.e. propofol)[32]. This indicates that lipids may be bioenergetically inert in critical illness[32]
Hypoxia and inflammation	• Critical illness is associated with hypoxia and inflammation and both can impair mitochondrial function and muscle protein synthesis[32] • Specifically, the activation of hypoxic and inflammatory signalling in acute critical illness have been directly related to impaired anabolic signalling

Reason	Details
	pathways[28,32]. In particular, several pro-inflammatory cytokines related to inflammation (e.g. CRP, IL-1, IL-6, TNF α) have been implicated in altered protein homeostatic signalling and loss of muscle mass[35]
Immobilisation and insulin resistance	• Immobilisation and muscle disuse (as is common in critical illness), induces muscle wasting. The mechanisms are complex and not fully understood; however, insulin resistance (which is associated with immobilisation) plays a key role in driving depression of protein synthesis[35]. • Stress hyperglycemia and insulin resistance are also common in early critical illness and thought to involve an adaptive mechanism that prioritises the utilisation of energy to vital tissues, further driving muscle loss[21]

The following section explores the clinical importance of skeletal muscle mass and quality in critical illness and outlines changes in muscle indices that have been observed in the literature. This is followed by discussion on body composition terminology and methodology (section 1.4) and nutrition assessment and therapy in critical illness (section 1.5).

1.3 Clinical importance of skeletal muscle in critical illness

Skeletal muscle is the most abundant tissue in the human body (40-50% total body weight), and with over 400 different skeletal muscles, makes up the majority of lean body mass[36]. In addition to skeletal muscle being important for the structural maintenance of the body and movement, it is also a metabolically active tissue with immunological properties, a critical reserve for amino acids to help with cell repair when needed, and plays an important role in influencing energy and protein metabolism[25,36]. The amount of skeletal muscle mass and also the quality of the muscle at ICU admission are thought to be important for recovery from critical illness and are discussed in more detail below.

1.3.1 Skeletal muscle mass and quality at ICU admission

The amount of skeletal muscle in an individual is influenced by age, hormones, physical activity, nutritional intake, and presence of any chronic diseases[37]. Specifically, increasing age, lack of physical activity, inadequate nutrition intake (particularly low protein), and presence of chronic diseases, can all contribute to reduced muscle mass[37].

The number of studies investigating the relationship between muscle mass at ICU admission and clinical outcomes has increased significantly over the past decade. This is primarily attributable to the emerging use of computed tomography (CT) scans performed for clinical diagnostic purposes for muscle assessment (*Note*: more detail on how CT scans can be used for body composition analysis is provided in section 1.4.1.2). In the critical care literature, a number of studies have utilised CT scans performed at or around ICU admission and have reported strong associations between low muscle mass and clinical

outcomes, including; in-hospital and 6-month mortality, ICU and ventilator days, and reduced likelihood of being discharged home[7-9,38,39]. This highlights the importance of identifying patients with low muscularity at ICU admission for prognostication and risk stratification and to identify those who may benefit from early intervention (e.g. intensive nutrition and/or physical therapy).

Similarly, in parallel to the emerging importance of muscle mass, a growing body of literature suggests that the quality of skeletal muscle at ICU admission may be important for recovery from critical illness. Muscle quality can be worsened by adipose and/or fibrous tissue infiltration into skeletal muscle[40]. The reasons for negative changes in muscle quality are not fully characterised, but are believed to be associated with increasing age, inactivity, and metabolic impairments (e.g. insulin resistance)[41]. Specifically in the critical care literature, low CT-measured muscle quality at ICU admission has been strongly associated with mortality at various time points including ICU discharge, six months, 180 days, and one year[42-44].

In addition to the amount and quality of skeletal mass at ICU admission being important for recovery, the changes that occur during the course of critical illness are also thought to impact recovery. The next section explores the magnitude of skeletal muscle mass and quality loss in critical illness reported in the literature using different body composition methods.

1.3.2 Changes in skeletal muscle *mass* during ICU admission

Early studies investigating lean tissue losses in critically ill patients used a specialised body composition technique, in vivo neutron activation analysis (IVNAA), to longitudinally measure changes in whole body protein[45-47]. These studies reported rapid and significant losses of whole-body protein of up to 12% by day 10 of critical illness[45]. Similarly, recent studies using muscle ultrasonography, have reported significant muscle loss of quadriceps musculature in acute critical illness. Specifically, reductions in rectus femoris cross-sectional area of 18-30% at day 10 have been reported[10-13].

As detailed above, significant muscle wasting during acute critical illness has been observed when IVNAA and ultrasound have been used to track changes in muscularity. However, when CT scans were utilised, findings were not consistent with those using other methods, with some studies reporting minimal to no change in skeletal muscle cross-sectional area (CSA) (mostly at the abdominal area) over the first few weeks of critical illness, whilst others reported marked losses. These studies using CT-image analysis are outlined in Table 1.3 below.

Table 1.3 Summary of studies investigating changes in CT-measured muscularity in critical illness

First author, year, country	n	Participant demographics	Intervention, study design	Outcome measure	Time of CT scans and time between scans	Skeletal muscle changes over study period
Bear 2021, UK[44]	n=54	Severe respiratory failure patients receiving venovenous extracorporeal membrane oxygenation (VV-ECMO) Age 46, Male 57%, BMI 28, APACHE II 18[a]	Retrospective	Abdominal muscle CSA, cm^2	CT1: within 24 hours of commencing VV-ECMO CT2: during ICU admission (median day 9 [7-18]) *Days between scans*: unclear. ~9 days (based on when the scans performed)	Overall median loss of 17.7% [-23.9% to -8.2%]
Braunschweig 2014, USA[48]	n=33	Respiratory failure patients Age 60±16, Male 61%, BMI 28±6, APACHE II 26±7	Prospective observational	Abdominal muscle CSA, cm^2	CT1: while in ICU CT2: any time during the hospital admission *Days between scans:* 10±5	Overall mean loss of 6.26%, (0.49% per study day), but did not reach statistical significance
Brewster 2014, Australia[49]	n=21	Acute pancreatitis Age n/a, Male 86%, BMI n/a, APACHE II 20	Retrospective	Abdominal muscle CSA, cm^2	CT 1: During ICU admission CT 2: During ICU admission *Days between scans*: 9.4 [7-19]	No significant difference between CT1 and CT2 (p=0.186)
Caesar 2013, Belgium[50]	n =15 *Early PN; n =10* *Late PN; n =5*	Neurosurgical *Early PN*: Age 44±16, Male sex 40%, BMI 24 [22-27], APACHE II 28 [26-32]; *Late PN*: Age 50±16, Male 40%, BMI 25 [23-26], APACHE II 30 [24-30]	Sub-study of a Prospective RCT[b]	Femoral and abdominal muscle CSV, cm^3	CT1: Median ICU day 2 [2-3] CT2: Median ICU day 9 [8-10] *Days between scans*: ~7	Mean loss of femoral muscle (6.9%±1.7%) No loss in abdominal muscle

Table 1.3 Cont.

Dusseaux 2019, France[51]	n=21	Medical ICU Age 64±11, Male 60%, BMI 28±6, APACHE II n/a	Retrospective	Abdominal muscle CSA at L3, cm^2. CSA was converted to 'skeletal muscle mass index', SMM = CT muscle CSA divided by height	CT1: within 48 hrs of ICU admission CT2: 7-14 days after CT1 *Days between scans*: 10.9±5	No significant difference between CT1 and CT2 (p = 0.183)
Yeh 2018, USA[52]	n=65	Surgical ICU Age 60±17, Male 49%, BMI 27±7, APACHE II 19±8[a]	Retrospective	Abdominal muscle CSA at L3 (Psoas muscle only), cm^2	CT1: within 72 hrs of ICU admission CT2: any time during the hospital admission *Time between scans*: n/a	Decrease from CT1 and CT2 in 63 (97%) of the patients The reported loss ranged from -11% to +0.8% per day. Mean loss and statistical significance was not reported

APACHE, acute physiology and chronic health evaluation; BMI, body mass index; CSA, cross-sectional area; CT, computed tomography; CSV, cross-sectional volume; ICU, intensive care unit; RCT, randomised control trial

Data presented as mean±standard deviation, or median [interquartile range]

[a]Data from overall cohort with baseline muscle measurement n=140, [b]Sub-study of the EpANIC trial[53]

As outlined in Table 1.3, most studies investigating changes in muscularity have utilised CT scans at the L3 region due to its relationship with whole-body muscularity (more detail and background on muscle assessment using CT scans is provided in section 1.4). Overall, the reported changes in CT-measured muscularity were highly variable ranging from no change to 17% loss over the study period (around 10 days). Comparison of the observed changes in CT-measured muscularity across the published studies is limited by the:

- Different muscle variables reported (e.g. total skeletal muscle CSA, psoas muscle CSA, skeletal muscle index [total muscle CSA divided by height in metres squared);
- Variable and often undefined time between CT scans; and
- Heterogenous study populations.

Furthermore, the precision of CT image analysis to detect changes in CT muscle CSA in critical illness is not known and may be influenced by fluid shifts (hence the differences observed in the above studies may not always represent real change). Chapter 6 aims to help address this knowledge gap, by assessing the short-term precision of CT image analysis to detect changes in CT-measured muscularity.

1.3.2.1 Changes in skeletal muscle mass over different phases of critical illness

As mentioned earlier in section 1.3.2, the majority of studies have focussed on changes in muscle mass over the first few weeks of critical illness. There is a paucity of data describing muscle changes in patients who stay in ICU beyond this time, likely due to challenges with prospectively identifying which patients are going to have a prolonged ICU stay, relatively smaller numbers of patients with long ICU stays, and the decreased need for CT scans for clinical purposes in those who do survive to stay a long time in ICU. However, research in this patient group is important as they have longer hospital admissions and are at much

greater risk of death and disability post ICU, and use disproportionally more health care resources compared to critically ill patients with shorter ICU stays[54,55]. Retrospective analysis using CT scans to explore changes in muscularity provides a unique opportunity to better understand the trajectory of muscle wasting throughout the different weeks of critical illness (as is explored in Chapter 6).

1.3.2.2 Changes in skeletal muscle mass and related outcomes

Skeletal muscle wasting observed in critical illness is believed to be a driver of the umbrella term "ICU-acquired weakness" (neuropathy and/or myopathy), which is common, and reported to develop in approximately 70% of critically ill patients[14]. ICU-acquired weakness has been independently associated with negative outcomes including short and long-term mortality, duration of hospitalisation, increased health-care costs, and reduced long-term physical function and quality of life[56,57]. A small number of studies have directly investigated the association between muscle mass loss and functional outcomes. For example, studies using ultrasound have reported associations between quadriceps muscle loss in the first 1-2 weeks of critical illness, and weakness and mobility at different time points (e.g., ICU discharge and 6 months post-ICU discharge)[10,12,13,58].

Whilst the loss of skeletal muscle mass early in the ICU stay is detrimental to long-term recovery, the restoration of muscle stores after critical illness is also likely to impact recovery. Specifically, in a study of 15 critically ill patients, all patients demonstrated significant quadriceps wasting 7 days following ICU discharge compared with published age- and sex-matched population-based norms[58]. By 6-months post-ICU discharge, quadriceps muscle was increased in all patients compared to the 7 days post-ICU

discharge measurement. However, there was significant variability in the extent of muscle repletion, with only 27% of patients having resolved quadriceps atrophy compared to the matched healthy population[58]. However, this regain of muscle did not correlate with resolution of weakness, owing to persistent functional impairment of the muscle[58]. These preliminary findings indicate that the loss of muscle over a very short period of time may have longer-term consequences for muscle strength and function.

1.3.3 Changes in skeletal muscle *quality* during ICU admission

As highlighted earlier (section 1.3.1), the quality of skeletal muscle mass may also be an important factor for rehabilitation after critical illness. Compared to skeletal muscle mass, the trajectory of changes in muscle quality during critical illness has been less well studied.

As discussed in more detail in section 1.4, measures of skeletal muscle quality can be obtained using ultrasonography (echogenicity) or CT-image analysis (skeletal muscle density, Hounsfield Units). Specifically, increased echogenicity indicates worsening muscle quality, whereas decreased CT-measured density indicates worsening muscle quality. Using ultrasound there have been differing reports of changes in quadricep muscle echogenicity over time. In a study of 25 ICU patients requiring extracorporeal membrane oxygenation, no significant change in muscle echogenicity was observed over the first 10 days[12]. In another study (n=22), quadriceps muscle echogenicity scores for rectus femoris and vastus intermedius increased over the first 10 days of critical illness by 13% and 26%, respectively (suggesting deterioration in muscle quality)[13]. Similarly, in a recent study of 41 critically ill patients with a diagnosis of sepsis or acute respiratory failure rectus femoris echogenicity increased by 10% if the first 7 days of critical illness[10]. This study also

reported that increased percentage change in echogenicity in the first 7 days of ICU admission was a strong predictor of ICU-acquired weakness at hospital discharge[10].

There are limited data using CT images to describe changes in skeletal muscle quality (density) in critical illness[50-52]. Two studies observing changes in the initial phases of critical illness reported no significant changes in CT-measured muscle quality[50,51]. Another study of 63 surgical ICU patients reported that 34 patients experienced a decrease in CT-measured muscle quality of the psoas muscle, whereas the remaining 29 patients had an increase in muscle quality[52]. In a recent study of 54 patients receiving venovenous extracorporeal membrane oxygenation, CT-measured muscle quality decreased by median 10.6% (IQR -23.6% to 13.4%) over approximately the first 10 days of ICU admission[44]. Limitations in comparing the small number of studies describing changes in CT-measured skeletal muscle quality include variable and undefined time periods between CT scans, and the heterogenous nature of patient cohorts. Furthermore, as highlighted in the following section 1.4 (which details the method for muscle quality assessment using CT scans), the administration and phase of contrast during CT scanning is known to influence measured values for CT-measured muscle quality, so it is imperative that comparator scans have comparable contrast administration[59]. It is not clear from the existing literature if contrast administration was considered and whether this may have contributed to the reports of improved muscle quality during the ICU stay, which is not consistent with prospective studies using ultrasound (although different muscle groups were investigated). Lastly, the precision of CT image analysis to detect changes in muscle quality is not known. Chapter 6 explores changes in CT-measured muscularity, while addressing the methodological limitations of previous studies.

As outlined above, both muscle depletion at ICU admission and detrimental changes in skeletal muscle mass and quality during critical illness have been associated with poorer recovery from critical illness. For these reasons, the quantification of skeletal muscle mass at ICU admission may be important for risk stratification and prognostication and also to evaluate strategies aimed at attenuating muscle changes during critical illness. There are additional reasons that the quantification of skeletal muscle mass may be beneficial in research studies and clinical management of critically ill patients, which are highlighted below.

1.3.4 Novel measure for diagnosing malnutrition?

International ICU nutrition clinical practice guidelines recommend early nutrition support for patients who are malnourished[17,18]. However, the evidence supporting these recommendations is of low quality (expert consensus)[18]. This may be explained by the fact that there are significant challenges in diagnosing malnutrition in the early days of an ICU admission, therefore making it difficult to test an intervention (e.g. early and intensive nutrition therapy) in this at-risk sub-population[60].

The diagnosis of malnutrition involves a number of components, including obtaining accurate anthropometric data, and weight and diet histories, all of which are difficult to acquire in the acute early phase of ICU admission when the majority of patients are bed-ridden and sedated[61]. Even if a weight is acquired, it may be inaccurate with many patients experiencing significant fluid shifts early in the ICU stay, which may mask muscle depletion. However, patients admitted for elective procedures may have an accurate weight taken just prior to ICU admission which is documented in the medical record.

As reduced muscle mass is highly related to malnutrition, the assessment of muscularity has been included as a key criterion in malnutrition diagnostic tools, such as the Global Leadership Initiative in Malnutrition (GLIM), Academy of Nutrition and Dietetics/American Society for Parenteral and Enteral Nutrition (AND-ASPEN), and the Subjective Global Assessment (SGA)[62-64]. Having a method to accurately and reliably detect individuals with low muscularity may provide an important indicator of malnutrition in critical illness (where the other strategies for diagnosing malnutrition are often not possible or are inaccurate), to enable further research ratifying the most appropriate nutrition management of this vulnerable group of patients.

1.3.5 To guide determination of nutrition requirements

As discussed in more detail in section 1.5, the delivery of nutrition to critically ill patients is most commonly guided by estimations of energy and protein requirements using weight-based prediction equations. Because of the issues with significant fluid shifts in critical illness, an estimated weight is most commonly used for input into these equations. With lean body mass being the biggest driver of metabolic rate, an objective quantitative measure of whole-body muscularity in the critically ill setting may help clinicians better determine nutritional needs. This is especially relevant in the context of increasing obesity in ICU populations and also where critically ill patients are admitted with pre-existing chronic illness influencing body composition where muscle mass relative to total weight may be low (e.g., chronic obstructive pulmonary disease, heart failure, chronic inflammatory disease).

1.3.6 Summary

This section has highlighted that recovery from critical illness is associated with baseline skeletal muscle mass and quality and changes in muscularity that occur in the first few weeks of ICU admission. The quantification of skeletal muscle mass may help to identify patients at risk of prolonged rehabilitation and determine nutrition requirements (and help to better target and evaluate nutrition therapy).

Hence, the development and evaluation of techniques to easily, reliably, and objectively provide estimates of whole-body muscularity at the bedside in both critical illness and in the post ICU phase, are imperative. Despite this, at the time of conceptualisation of the research studies for this thesis there was no validated method for assessing muscularity at the bedside in critically ill patients, and this had been identified as a key critical care nutrition research priority[65].

The next section provides an overview of the study of body composition; including terminology, models, and methodology, with a focus on the application of methods in critical illness.

1.4 Body composition: an overview of terminology and models

The composition of an organism reflects lifetime accumulation of nutrients and other substrates acquired from the environment and retained by the body[66]. These components range from elements to tissues and organs, and are the building blocks that give mass, shape, and function to all living things[66]. Human body composition analysis techniques allow clinicians and researchers to observe and study how these components function and change with age and metabolic state, and to diagnose conditions related to health, function, and health risk, and evaluate changes in response to interventions[66].

1.4.1 Body composition research

The study of body composition has been described as three interconnected areas: rules and models, methodology, and variation (Figure 1.2)[66]. These areas will be discussed in more detail in the sections below.

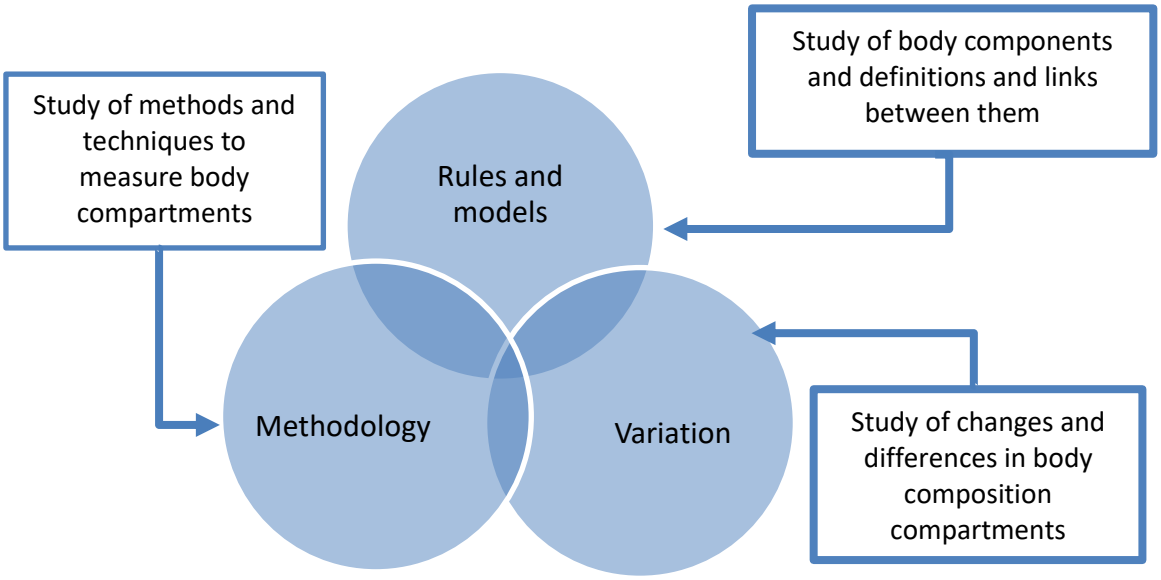


Figure 1.2 Three interconnected areas of body composition research, adapted from Wang et al[67]

1.4.1.1 Body composition rules and models

The study of body composition rules and models involves investigation of the components themselves, definitions, and links between them[66]. There are around 30-40 major body components, including those that represent component combinations at different levels[66]. When combined in a mathematical fashion they are referred to as models[66]. A classic model is the prediction of fat-free mass (FFM, kg) from total body water (TBW, kg), with the common formula being FFM = TBW divided by 0.732[68]. This model assumes that FFM is 73.2% water, which has been shown to be stable under healthy conditions in adults. However, this assumption is not constant across the lifespan (varies with age) and is often violated under clinical conditions when hydration may be altered (e.g. critical illness) and in extreme obesity[68]. Other models assume a consistent relationship between regional measurement of muscularity and whole-body muscularity. For example, the quantification of skeletal muscle using a single-slice computed tomography (CT) scan at the third lumbar area has been used in a range of clinical conditions to provide an estimate reflecting of whole-body muscularity[68].

Within body composition research there are frameworks for describing the relationships between major body compartments, to help clarify what body compartment is being measured by a particular body composition technique[66]. The most widely accepted framework in body composition research divides the human body into five different levels: atomic, molecular, cellular, tissue-system, and whole body[67]. Common levels used in the modern-day clinical environments are: whole-body, molecular, and tissue-organ[69]. Figure 1.3 outlines these levels and indicates the compartments that are measured by commonly used body composition modalities.

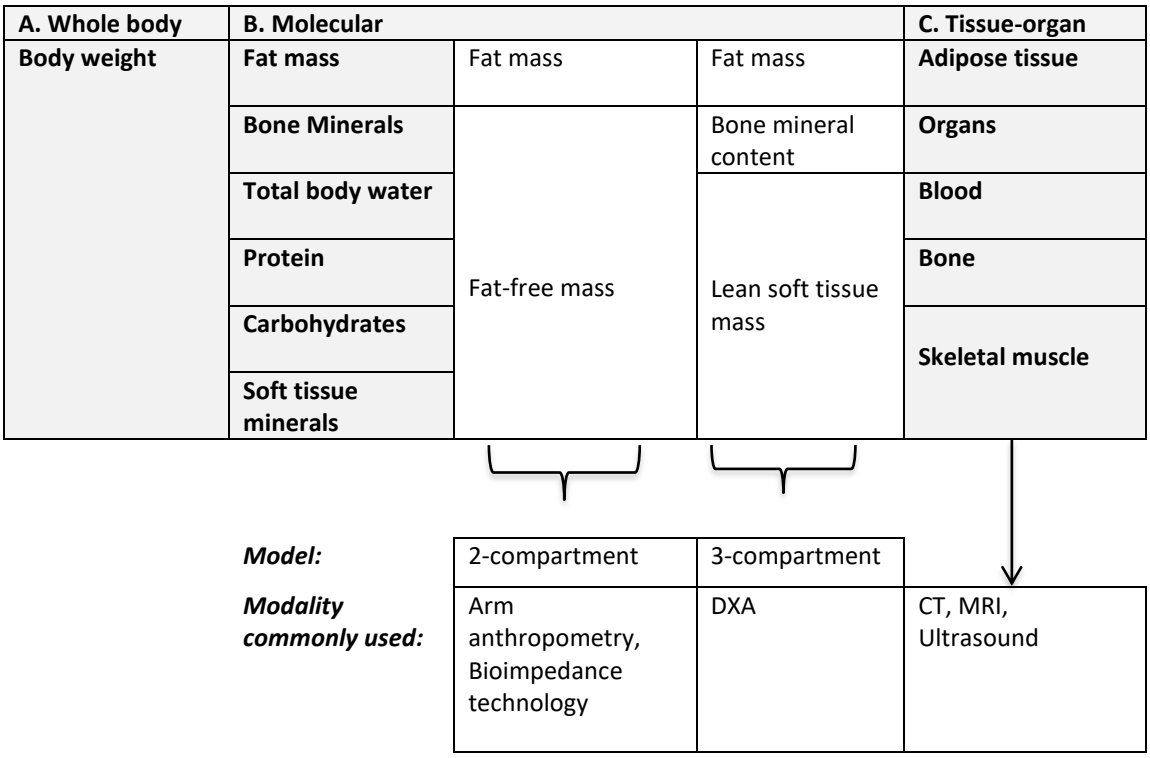


Figure 1.3 Body composition framework, outlining levels and compartments that are measured by commonly used body composition modalities. CT, computed tomography, DXA, dual-energy x-ray absorptiometry; MRI, magnetic resonance imaging. Adapted from Prado et al[69]

As indicated in Figure 1.3, the molecular level can be further defined into the two-compartment (defined as fat mass and FFM) or the three-compartment model (fat mass, bone mineral content, lean soft tissue mass). This includes techniques where the raw measurement is imputed into a prediction equation to derive the whole-body level compartment (e.g. bioimpedance algorithms generate total body water and then FFM from height and raw bioimpedance data, such as reactance and resistance)[68].

At the tissue-organ level, techniques quantify specific body parts (e.g. skeletal muscle) but prediction equations developed to estimate whole-body compartment, are in earlier stages of use and validation (e.g. CT, ultrasound), and not yet ready for use in clinical

practice[66]. It is important to understand and consider how the techniques derive estimates of whole-body values because 1) conversions of regional measurements to whole body values rely on underlying assumptions which may or may not hold true, particularly in the clinical setting (i.e. where regional muscle depletion may be present, for example lower limbs in late critical illness); and 2) to ensure appropriate interpretation and comparison of studies when different compartments are measured.

The following section provides an overview of body composition methodology and techniques commonly used in the clinical setting. Following this, due to the clinical importance of skeletal muscle in critical illness (as outlined in section 1.3), the focus moves to the validation of methods for muscle assessment the ICU setting.

1.4.1.2 Body composition methodology

The second area of body composition research involves body composition methodology. There are many methods and techniques available to measure the major body components of the five levels *in vivo* and *in vitro*, all with variable measurement precision, reliability, safety, cost, portability, accessibility, expertise, level of patient burden, and equipment required for measurement[69]. Body composition modalities include[69]:

- Anthropometry
- Hydrodensitometry/underwater weighing and air displacement plethysomography
- Dilution tracer techniques
- Whole-body potassium counting and neutron activation analysis
- Bioimpedance analysis
- Ultrasound
- Dual-energy x-ray absorptiometry (DXA)
- Magnetic resonance imaging (MRI)
- Computed tomography (CT)

Table 1.4 provides a summary of commonly used body composition techniques used in clinical settings and briefly highlights the applicability of each method in the ICU setting. Further discussion about each method and use in critically ill patients is provided in subsequent sections.

Table 1.4 Summary of commonly used body composition techniques used in clinical settings and applicability in ICU, adapted from[68,69]

Technique	Method details	Low cost	Safe*	Validity/ Accuracy	Applicability to ICU setting
Reference techniques					
DXA	Uses very low radiation x-rays of two beams of energy. The generation of high- and low- energy emission by an x-ray source is used to differentiate between soft tissue and bone. Fat mass is then estimated from specific attenuation characteristics of soft tissues.	x	✓	✓	• Often not possible or appropriate to send critically ill patient to DXA machine for body composition assessment
CT	The x-ray attenuation through tissue is detected and an image is constructed. Skeletal muscle can be quantified by using the known x-ray attenuation (density) of muscle and specialised software.	x	x	✓	• Can be utilised in ICU when scans performed for clinical purposes • Requires specialist training and software to quantify body compartments
Bedside techniques					
Anthropometry Lengths, circumferences, skin folds	Uses tape measures and skinfold calipers. Provides surrogate assessment of muscularity which can be compared to population-based data (muscle mass not directly measured). Estimates of fat-free mass and muscle size can be obtained through equations.	✓	✓	?	• Unclear reliability and precision in the ICU setting where the technique may be impacted by fluid overload and measurement (positional) challenges • Methods are relatively insensitive
Impedance Bioimpedance analysis	Measures the resistance and reactance from the application of a weak alternating electrical current applied to the body. The differential flow of the current depends on the body composition (e.g. muscle and blood easily conduct the current, whereas bone and fat do not). An estimate of total body water is provided which is then used to estimate fat mass and fat-free mass.	✓	✓	?	• Relies on population-specific equations, which assume normal hydration (some not released by manufacturers). • Unclear precision in the ICU setting where patients often experience significant fluid shifts and subject conditions are not ideal (e.g. not fasted)
Ultrasound	Ultrasound beam is transmitted through the skin and acoustic waves are reflected from the tissue back to the transducer. The amount of reflection is based on the changes in acoustic impedance which is different among air, fat, muscle, and bone.	✓	✓	?	• Ultrasound devices available in most ICUs • Unclear reliability and precision to provide a surrogate measure of whole-body muscularity in the ICU setting • Limited “cut-points” to determine individuals with low muscle mass using ultrasound

CT, computed tomography; DXA, dual-energy X-ray absorptiometry; ICU, intensive care unit. *No-low radiation

The following sections will focus on the validation of techniques to assess muscle mass in critical illness.

Terminology

The majority of the techniques used and referred to in this thesis measure skeletal muscle so the term *'skeletal muscle'* and *'muscularity'* will be used as a general term throughout to describe indices of muscle or lean mass.

Validation of bedside techniques to assess muscularity in clinical settings

With exception of cadaver analysis, all body composition techniques are indirect, requiring multiple assumptions that may or may not be violated in acute and/or chronic illness, and none are completely free from error[68]. Hence, the term *reference method* has been used in this thesis in preference to *gold standard* to describe the more established techniques which have more data supporting their accuracy and reliability, and against which bedside methods can be evaluated and/or validated[68]. Of note: this thesis will focus on concurrent validity – specifically the relationship between muscularity assessed by a bedside methods compared to a reference method. An additional approach to validate the clinical utility and application of bedside techniques includes investigating the relationship between muscle assessment and clinical and patient-centered outcomes (predictive validity). While both are important, this thesis focused on concurrent validity.

Terminology regarding validity

The overall aim of determining if a bedside body composition method is a 'valid' method to assess muscularity is to establish that the method is acceptable for use in the intended setting, often because a reference method is more expensive, technical or less widely

available[68]. As detailed by Earthman, when investigators are aiming to validate a device or technique (i.e. to determine acceptability) the following three points should be considered[68]:

1) How closely the values obtained by the bedside method agree with the values obtained by the reference method (at the individual and group level);
2) How often the values are within an acceptable range of difference (at the individual and group level); and
3) Whether there is a consistent tendency for the bedside method to over- or underestimate the body composition compartment of interest compared with the reference method (acknowledging that reference methods also have limitations, e.g. can be impacted by overhydration).

To address these issues, it is important to evaluate the precision, accuracy, and bias of the bedside method to measure the variable of interest. Table 1.5 outlines the key terminology, and examples of the statistical methods employed to assess each.

Table 1.5 Key terminology for determining the validity of a body composition technique[68]

Concept	Definition	Examples of statistical method
Precision and reliability	• Degree of agreement among repeated measurements (i.e. how well does a particular method produce the same result on multiple occasions, and encompasses inter- and intra-rater observer reliability)	• Coefficient of variation • Intraclass correlation coefficient • Test-retest correlation coefficients (same day or between day)
Accuracy	• Closeness of agreement in a variable measured with two assessment methods (i.e. how close are the values of a variable by a bedside method compared to those from the reference method) • Important to consider accuracy at both individual and group level as they are not quite the same (i.e. group level accuracy is good but may not be sufficient for clinical setting)	• Correlation analyses • Linear regression • Paired t-test • Bland-Altman analysis • Standard error of estimate • Concordance corelation coefficient
Bias	• Systematic error in a method (i.e. the difference between the measurements made by the bedside method and those by the reference method)	• Mean (± standard deviation) of differences

As highlighted earlier (section 1.3), low muscularity has been associated with a worsened recovery from critical illness and malnutrition. Therefore, an additional consideration when evaluating the usefulness of a bedside method is whether the device or method can correctly identify those individuals with lower-than-normal muscularity as assessed by the reference method. The terms *sensitivity* and *specificity* are often used when determining the diagnostic ability of a method to identify the individuals of interest, for example those

with low muscularity via the reference method[68]. Sensitivity can be defined as the percentage of individuals with low muscularity who are correctly identified using the bedside method. Specificity can be defined as the percentage of individuals with normal muscularity correctly identified by the bedside method as not having low muscularity[68]. If a bedside body composition technique is to be clinically useful to identify patients with low muscularity it should have high sensitivity and specificity (ideally >90%, especially for sensitivity)[68].

While sensitivity and specificity of a test are defined as the proportion of people with the condition who will have a positive or negative test, positive and negative predictive values (PPV and NPV) can be used to describe the odds of a patient having a condition if they have a positive or negative result, respectively[70]. Specifically, PPV represents the percentage of patients with a positive test who actually have the disease/condition (i.e. low muscularity) (PPV = true positive/ [true positive + false positive])[70]. NPV represents the percentage of patients with a negative test who do not have the disease (NPV = true negative/ [false negative + true negative])[70].

Another statistical approach often used to assess the accuracy of a diagnostic test/device/method is receiver operator characteristic (ROC) curve analysis, which plots the true positive rate (sensitivity) against the false positive rate (1- specificity)[71], see Figure 1.4. In general, an area under the curve (AUC) value of 0.5 suggests no discrimination (no diagnostic ability), 0.7 to 0.8 is considered acceptable, 0.8-0.9 is considered excellent and more than 0.9 is considered outstanding (perfect diagnostic ability)[71].

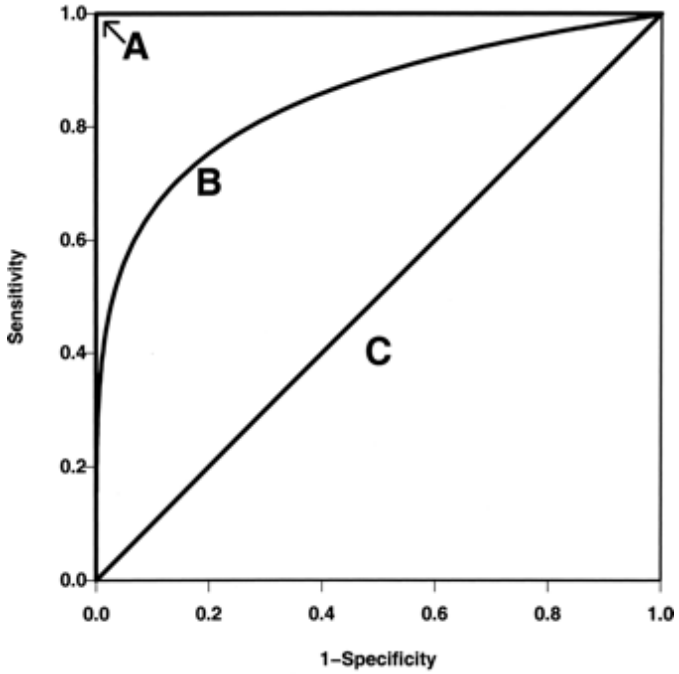


Figure 1.4 Three hypothetical ROC curves representing accuracy of the reference method (line A; AUC=1), acceptable alternate method (line B; AUC=0.85), and a useless method (random chance, line C; AUC=0.5)*

The following section outlines the reference and bedside methods for consideration in clinical populations.

* *Reproduced from Zou et al. (2007). Receiver-operating characteristic analysis for evaluating diagnostic tests and predictive models. Circulation, 115(5), 654-657, with permission from Wolters Kluwer Health, Inc.*

1.4.1.2.1 Reference methods

Modern reference body composition techniques used in clinical populations include DXA, MRI, and CT. DXA is the reference method most commonly used in the research setting because it provides a quick, non-invasive, and safe (low-level radiation exposure) whole body measurement of body composition[68]. However, DXA scanners are neither readily available nor practical for use in many clinical settings. Specifically, critically ill patients are often receiving organ support and it is often not appropriate or possible to transport a patient to specialised machinery for body composition assessment, nor undertake the measurement (i.e. cannot transfer patient onto scanner when so critically unwell).

MRI and CT scans have been commonly used for body composition analysis in a range of clinical conditions[68]. These techniques use strong magnetic fields (MRI) or high dose radiation (CT) to produce high quality axial cross-sectional images, from which skeletal muscle can be quantified by a trained investigator/clinician by using specialised software[68]. Due to the cost, time, transport, and radiation involved (CT), body composition analysis has typically only been an option when the scan has been performed for clinical diagnostic purposes. It is important to note that this opportunistic use of these scans limits the populations where evaluation of body composition can occur and the availability is likely to vary around the world and in different clinical settings. For example, in the United Kingdom patients receiving extracorporeal membrane oxygen will routinely be sent for a baseline CT scan, whereas in Australia this is not routine practice.

Whole-body MRI or CT scans performed for diagnostic purposes are not performed as widely as regional scans, which often include the abdominal area. This led to the investigation of whether cross-sectional assessment of muscularity is indicative of whole-

body values. In 2004 Shen et al investigated the agreement between skeletal muscle CSA from a single MRI scan at various anatomical landmarks in the abdominal region and whole-body muscle from full body MRI scans in a group of healthy volunteers[72]. They found the strongest associations from scans around the L3 area, a result which has since been replicated in oncology populations[73]. A single slice analysis at the L3 region is now a commonly used landmark to assess muscularity in specific clinical populations (primarily oncology) and has excellent precision when specialist software is used by trained investigators[68]. Figure 1.5 displays muscle mass assessment at L3 using a single slice CT image. In the ICU setting, MRI scans are not regularly performed for clinical purposes. However, CT scans including the L3 area are routinely performed for some ICU populations (e.g. trauma patients), and used for clinical monitoring and investigation (e.g. complications post gastrointestinal surgery) enabling the investigation of muscularity in these patients.

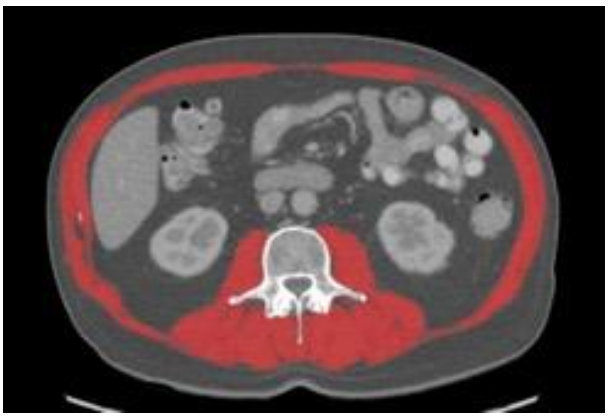


Figure 1.5 A single CT slice at the third lumbar (L3) area*. Skeletal muscle cross-sectional area, is indicated in red.

* *Image taken from study patient as part of one the research studies for this thesis*

Use of CT scans for muscle assessment in critical illness

The majority of studies utilising CT scans for muscle assessment in the ICU literature have recruited patients who had a scan at L3 performed on or around ICU admission and investigated the association between CT-measured muscle mass and/or quality and clinical outcomes (discussed in Section 1.3). There are a number of small studies that aimed to describe changes in muscularity at the abdominal region using CT scans, with differing results (also outlined in Section 1.3 and Table 1.3). At the time of the conceptualisation of the research studies in this thesis, to the candidate's knowledge, there was only one study (VALIDUM study) which had used CT image analysis as a reference method to evaluate the relationship with bedside assessment of muscularity (using ultrasound)[74]. This study is discussed in more detail in Section 1.4.1.2.2.

CT scans can also be used to measure the density of skeletal muscle and/or intramuscular adipose tissue (usually done at L3 area), with both variables being used in the literature as a surrogate measure of muscle *quality*. The administration of contrast during CT scanning can influence the muscle density measurement and is an important consideration when observing changes in muscle quality over time[59,75]. Figure 1.6 displays CT scans of two individuals with the same BMI but differing body composition - one with high quality muscle (low intramuscular adipose tissue and high muscle density) and the other with low quality muscle (high intramuscular adipose tissue and low muscle attenuation).

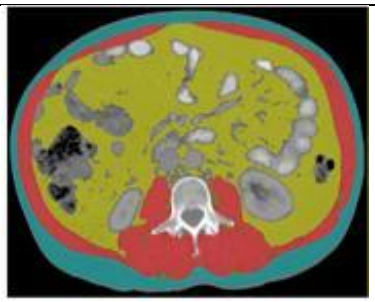
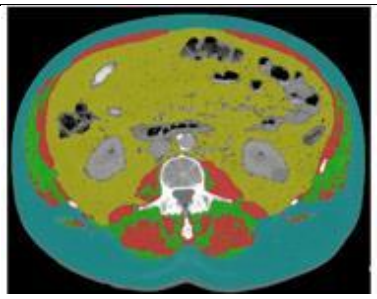
	High muscle mass and quality	Low muscle mass and quality
		
Age, years	58	77
BMI, kg/m^2	32	32
Skeletal muscle CSA, cm^2 (red)	194	114
Intramuscular adipose tissue, cm^2 (green)	2	78
Muscle density, HU	42	12

Figure 1.6 Two individuals with differing body composition as assessed by CT image analysis*. BMI, body mass index; CSA, cross-sectional area, HU, Hounsfield units.

Identifying patients with low muscularity using CT scans

In 1998, Baumgartner et al investigated the relationship between DXA-measured low muscularity and functional outcome and disability in a large cohort of elderly Hispanic and non-Hispanic men and women living in Mexico (n=883)[76]. In this study, sex-specific height-adjusted cut-points for low muscularity were defined as the value two standard deviations below the mean derived from a cohort of healthy adults (mix of African-Americans and Caucasians, n=284)[77]. The prevalence of low muscularity increased significantly with age from 14-24% in persons aged <70 years to 60% in persons aged >80 years of age[76]. Furthermore, the authors reported for the first time that low muscularity was significantly associated with self-reported disability in both men and women, independent of ethnicity, age, obesity, income, and health behaviours[76].

*Images displayed with permission from Lisa Murnane (Dietitian and PhD candidate, The Alfred hospital). The images were analysed as part of one of her PhD research studies

Similar approaches were subsequently taken using CT scans. Specifically, in 2008 Mourtzakis et al, aimed to compare estimates of whole-body composition obtained by single-slice CT with those obtained by DXA in a cohort of patients with locally advanced or metastatic non-small cell lung or colorectal cancer[73]. The mean age of all patients recruited was 63±10 years and mean BMI was 26.9±6.2kg/m^2. CT muscle CSA at L3 was found to be strongly related to appendicular lean tissue mass measured with DXA (n=31)[73]. Using the existing DXA-derived cut-points for low muscularity applied in the Baumgartner et al study, they reported corresponding sex-specific cut-points for low CT skeletal muscle index that were adjusted for height (muscle CSA divided by height in metres squared; 55.4cm^2/m^2 for males and 38.9cm^2/m^2 for females)[73]. These CT cut-points for defining low muscle mass, as well as others developed based on mortality data (e.g. Prado et al who recruited a large oncology population, 52.4cm^2/m^2 males and 38.5m^2/m^2 females[78]), have since been used extensively in the oncology literature as a predictor of clinical outcomes.

Low muscularity at ICU admission and relationship with outcome

A number of studies investigated the relationship between low CT-measured muscularity at ICU admission and clinical outcomes. Some cut-points used to identify patients with low muscularity were defined based on mortality data, while others have used existing cut-points derived from oncology populations. For example, in 2014 Weijs et al recruited 240 general ICU patients who had a CT scan at ICU admission[7]. Using receiver operator characteristic (ROC) curve analysis, sex-specific CT muscle CSA cut-points best fit to predict hospital mortality were identified (CSA <170cm^2 males, and CSA <110cm^2 females)[7]. In this study, 63% of patients (for both males and females) were classified as having low CT muscle CSA[7]. Other studies used existing cut-offs derived from cancer patients. Moisey et

al recruited 149 elderly trauma patients (mean age 79 years, range 72-85 years) who had a CT scan at ICU admission and used the Mourtzakis et al. CT muscle index cut-points discussed earlier to identify patients with low muscularity (55.4cm^2/m^2 male and 38.9cm^2/m^2 female)[8]. The authors reported a high prevalence (71%) of patients with low CT muscle CSA[8]. As the loss of muscle mass with aging has been well-described, it is not surprising that in this elderly cohort that there was a high proportion of patients identified with low muscularity using cut-points derived from a younger cohort. Despite this, the patients who were identified as having low muscularity at ICU admission in this study had higher in-hospital mortality compared to those with normal muscularity (32% versus 14%; p=0.018)[8]. Furthermore, multivariate linear regression demonstrated that low muscularity using the cut-points (but not muscle index as a continuous variable) was associated with fewer ventilator-free days (p=0.004) and ICU-free days (p=0.002)[8]. This indicates that these cut-points derived from a younger oncology cohort may have applicability in critically ill populations.

Other studies have generated cut-points for low muscularity using optimum stratification based on mortality data. For example, in a group of 99 cancer patients admitted to an ICU, Toledo et al compared CT-derived muscularity to BMI and determined its relationship with 30-day mortality[79]. Cut-points for low muscularity (based on 30-day mortality) were defined as 55.27 cm^2/m^2 in males and 40.13 cm^2/m^2 in females[79]. These findings are not dissimilar to the cut-points described earlier derived from the non-critically ill cancer population[73].

In summary, there are currently no universally accepted cut-points to define low muscularity in critically ill patients. Due to the heterogeneity of critically ill populations, it is unlikely that a single set of cut-points will be applicable to all patients admitted to an ICU. When evaluating muscularity status using existing cut-points, consideration of how representative the reference population is to the study population is likely to be a critical consideration (i.e similar ethnicity, age, and clinical condition).

Limitations and challenges with CT image analysis in critical illness

Although CT images are highly sought after for their accuracy and precision in quantifying muscle mass, there are a number of practical limitations such as cost, availability, and radiation exposure. As discussed earlier, this limits the use of this method to those patients having a CT scan performed for clinical/diagnostic purposes and those clinically determined timepoints. This often precludes the prospective assessment of longitudinal changes in muscularity at pre-defined intervals. Furthermore, assessment is time-consuming and requires specialist training, both of which limit the use of CT image analysis as a tool for clinicians at the bedside. For these reasons, the development of portable, non-invasive, and easily applied methods to measure or estimate muscularity at the bedside in the ICU setting is a key research priority[65].

1.4.1.2.2 Bedside methods for consideration in critical illness

As the majority of critically ill patients are bed-ridden, any method for bedside assessment of muscularity must be able to be easily and reliably implemented in the supine position, with little or no input from the patient. The following section highlights potential methods for investigation in the ICU setting.

Ultrasound

Ultrasound is used routinely in clinical practice for diagnosing and treating a variety of diseases and conditions. Ultrasound devices non-invasively produce high frequency sound waves (1-14 MHz), that are generated by the vibrations of electrically stimulated piezoelectric crystal within the head of the transducer, producing ultrasonic waves[80]. When acoustic coupling gel is applied to the transducer, these ultrasound waves transmit through the skin and are then partly reflected and partly transmitted through the underlying tissues[80]. The level of reflection and transmittance that occurs is dependent on changes in acoustic impedance as well as the characteristics of the underlying tissues[80]. The waves that are reflected back to the transducer are received by the piezoelectric crystals and processed based on timing, frequency and amplitude of the reflected waves which is then displayed as a 2-dimensional image on the ultrasound screen[80].

For decades, ultrasound has been used for body composition assessment, firstly as a technique to measure and assess subcutaneous adiposity, followed by muscle tissue assessment in the sports injury and neuromuscular disease fields[66,68]. More recently, ultrasound has emerged as a promising bedside tool to quantify and track changes in skeletal muscle in critically ill patients[68]. The method shows great potential as a widely applicable tool in the ICU with its accessibility (most ICUs have a device), as well as its relatively low cost, portability, and ease of use once trained[81].

Using ultrasound to assess muscularity

Ultrasound can be used for estimating muscle mass by acquiring transverse cross-sectional images at predefined anatomical landmarks and then analysing those images for the thickness or CSA of the underlying muscle group(s) (Figure 1.7). The muscle thickness

or CSA measures, in conjunction with prediction equations, can give estimates of whole body or regional measures of lean tissue or muscle mass[82-84]. However, these prediction equations have not yet been well validated in clinical populations as discussed in more detail in below.

(A) (B)

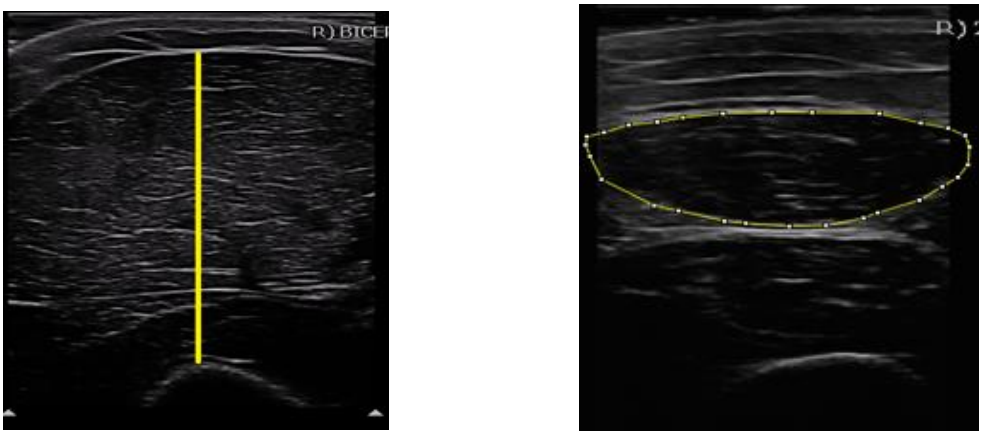


Figure 1.7 Example ultrasound image taken for muscle assessment*

(A) Vertical yellow line indicates mid-upper arm muscle thickness (cm) from superficial fat-muscle interface to the humerus, (B) Circular yellow line indicates quadricep rectus femoris CSA (cm^2)

While muscle CSA may provide a more detailed analysis of muscle architecture compared to muscle thickness, the thickness measurements are more commonly used for lean tissue mass estimations because the analysis is less time consuming (i.e. it is easier to measure from the bone to the inner muscle fascia layer compared to the entire fascia of the muscle). Additionally, for many muscle groups, such as the rectus femoris, the entire muscle CSA may be too large to visualise with a standard linear ultrasound probe. Furthermore, cut-points for identifying individuals with lower-than-normal muscularity

* *Images taken from study participants enrolled into the prospective observational study as part of this thesis.*

using ultrasound-derived muscle thickness have been recently reported, further progressing this method for muscle assessment[85].

Existing protocols for assessing muscularity using ultrasound

A number of ultrasound protocols have been developed to measure either muscle thickness or CSA of a wide range of muscles using many different landmarks[86]. The majority of ultrasound protocols have been applied in healthy volunteers and involve measuring muscle thicknesses at multiple sites (commonly seven to nine anterior and posterior sites) while the patient is in a standing posture[82-84,87]. Many protocols in the ICU literature (as highlighted in section 1.3), have measured muscularity at the quadriceps by measuring muscle thickness using a four-site protocol (two sites on bilateral quadriceps)[13,88-90], or rectus femoris CSA, while the patient is supine[10,11,13.]

Despite the increasing use of ultrasound to measure muscularity and track changes over time in the ICU literature, only the nine-site protocol has been extensively validated for lean tissue or muscle mass estimations in healthy volunteers, being compared to hydrostatic weighing[83], MRI[84,91] and DXA[82,92] measures of fat-free, lean tissue or muscle mass (regression co-efficients ranging from 0.75-0.98)[86]. Many of these studies also reported sufficiently narrow limits of agreement when Bland-Altman analysis was performed when compared with either DXA lean tissue mass or MRI muscle mass. Furthermore, there were no observable biases in the cross-validation groups within any study[86]. However, while the studies developing regression equations did not observe any bias, when four previously published regression equations developed in Japanese populations were applied in a cohort of older Caucasian adults, significant systemic and proportional bias was observed in three of the four equations[93]. Overall, these findings

indicate that there is strong agreement between the nine-site ultrasound protocol and DXA or MRI measures of lean tissue or muscle mass at a group level; however, the regression equations are likely to be specific to the population in which they are developed in[86].

While the nine-site ultrasound protocol may provide accurate estimations of muscularity, it is not feasible to perform in many clinical populations including in critical illness, where the patients are generally unable to stand. In healthy volunteers, a few studies have compared ultrasound protocols including landmarks accessible while individuals are in the supine position against reference measures of muscle mass. One study in 36 volunteers found that the sum of thicknesses of anterior upper arm, anterior forearm and anterior upper leg, obtained in the supine position was strongly associated (r=0.87) with lean tissue mass assessed by DXA[94]. In a recent study of 96 healthy volunteers, three different ultrasound protocols were compared with appendicular lean tissue mass assessed by DXA (nine-site, four-site [bilateral quadriceps], and five-site [bilateral quadriceps and anterior mid-upper arm])[85]. Muscle thicknesses were multiplied by limb length. The four-site protocol had a strong relationship to appendicular lean tissue mass (R^2=0.72), which was further improved by adding anterior mid-upper arm muscle thickness and covariates age and sex (R^2=0.91), with a standard error of the estimate of 1.62kg[85]. These results were identical to those obtained from the nine-site protocol. Furthermore, using the optimised five-site protocol (five-sites + age + sex), Bland-Altman analysis revealed limits of agreement from -3.18 to 3.18kg, which the authors reported was acceptable based on the average appendicular lean tissue mass difference in a large cohort of Caucasian older adults[85,95]. Overall, these findings indicate the possibility for ultrasound at the upper and

lower limbs to provide a quantitative estimate of whole-body muscularity in clinical conditions where patients are bedridden.

Measurement considerations - compression technique and reliability

The majority of existing studies used a minimal compression technique while taking ultrasound images. This involves applying a large amount of water-soluble gel to the transducer and minimally compressing the skin, ensuring that there is no tissue depression on the image[85]. In clinical states where fluid overload is common (i.e. critical illness and liver failure), maximal compression ultrasound technique was proposed to limit the impact of fluid on muscle assessment. Maximal compression involves maximally compressing the underlying tissue with the transducer[74]. Figure 1.8 demonstrates ultrasound images when maximal and minimal compression technique are applied.

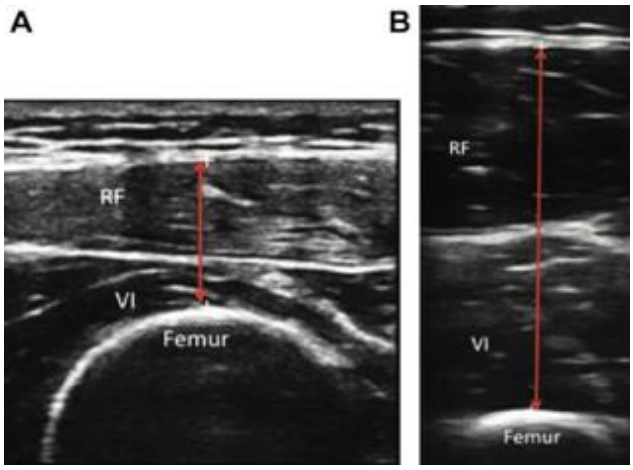


Figure 1.8 Thigh ultrasound demonstrating (A) maximal compression and (B) minimal compression*. Muscle thickness is indicated by the red arrow. RF, rectus femoris; VI, vastus intermedius.

* *Reprinted from Tandon et al (2016). A model to identify sarcopenia in patients with cirrhosis. Clinical Gastroenterology and Hepatology, 14(10), 1473-1480, with permission from Elsevier.*

In the aforementioned Paris et al study in 96 healthy volunteers, the minimal compression technique was compared with maximum compression (reliability testing and correlations to DXA measured lean tissue)[85]. The authors reported appropriate intra-rater reliability for both compression techniques: minimal = CV 1.1% and ICC 0.988 [CI 0.996, 0.998] versus maximal = CV 2.5% and ICC 0.989 [0.983, 0.993][85]. However, with inter-rater reliability, the minimal compression technique was superior: CV 3.7% and ICC 0.988 [0.966, 0.996] versus CV 9.0% and ICC 0.945 [0.843, 0.981] for maximal compression[85]. Furthermore, there were stronger correlations with lean tissue mass when using minimal compression (R^2=0.82), compared with maximal compressions (R^2=0.66), at the site-specific landmarks using DXA[85].

In another study investigating 159 outpatients with cirrhosis, a four-site ultrasound protocol measuring muscle thickness at the bilateral quadriceps was compared to muscle mass assessed by cross-sectional images from CT or MRI analysis, using both minimal and maximal compression[96]. The minimal compression technique was associated with significantly lower inter-rater variability than the corresponding maximal compression assessment (Pearson correlation= 0.97 versus 0.85; and intraclass correlation= 0.99 [95% CI 0.97-0.99] versus 0.92 [95% CI 0.85-0.95])[96].

Specific to ICU, a recent study by Fetterplace et al, compared a four-site ultrasound-protocol (bilateral thighs) using maximal and minimal compression to CT muscle CSA at L3[97]. The ultrasound muscle thickness measurements were obtained from an earlier prospective randomised control trial[90] and were retrospectively compared to CT-measured muscularity if the participant had a CT scan performed at L3 area within 72 hours of the ultrasound measurements. There were 35 patients included, with 33

occasions where the CT scan was performed at ICU admission and eight occasions where the CT scan was performed at a later period. The median time between the ultrasound measurements and CT scan was 43 hours [interquartile range 38 – 46 hours])[97].

When adjusted for sex, age, and body mass index, bilateral thigh muscle thickness measured using the maximal pressure technique remained a strong independent predictor of lumbar muscle CSA[97]. Specifically, the mean skeletal muscle CSA was estimated to be greater by 35cm^2 (95% CI = 11 to 59), for each additional centimetre observed in thigh muscle thickness[97]. Despite these findings, the authors reported substantial uncertainty within all regression estimates[97]. Although broadly similar associations were observed for measurements obtained using the alternative minimal compression technique, the strength of these relationships was lower[97]. It is important to note that this study has a number of methodological limitations. As highlighted in section 1.3, muscle wasting at the quadriceps musculature occurs rapidly in critical illness, hence a critical comparison between two assessment methods (especially where one is measuring skeletal muscle at the abdominal area), should ideally occur with a short period between the two methods (i.e. <24-48 hours) and at ICU admission. Although this recent retrospective study adds to the literature in demonstrating potential for ultrasound at the thighs to provide a quantitative assessment of muscularity, the strength of the relationship between the two methods is likely to be influenced by the extended time period between muscle assessment and the fact that 20% of measurements were taken later in the ICU admission[97]. This highlights the challenges with performing these type of 'validation' studies retrospectively. It is also challenging to interpret the difference in maximal and minimal pressure findings reported in this study where the sample size is

small, there are wide regression estimates with both techniques, and no reliability testing was reported.

Integrating the literature, it appears that the minimal compression technique is more favourable in terms of reliability and shows a stronger relationship with muscularity assessed with a reference method than maximal compression. For this reason, minimal compression ultrasound technique was used in the study described in Chapter 3.

Validation of ultrasound against a reference method in critical illness

There is a paucity of studies comparing muscularity assessed by ultrasound and a reference method in the ICU setting. At the time of developing the research studies for this thesis, there was only one published study that compared ultrasound-measured muscularity to CT-derived skeletal muscle CSA at the L3 region at ICU admission (Validation of Bedside Ultrasound of Muscle Layer Thickness of the Quadriceps in the Critically Ill Patient, VALIDUM)[74]. The VALIDUM study was a prospective, multi-centre observational study of 149 patients who had a CT scan performed for clinical purposes <72 hours after ICU admission. The ultrasound protocol included measurement of muscle thickness at bilateral quadriceps (four-site protocol), using maximal compression[74]. In VALIDUM, the ultrasound protocol was performed a mean of 1.1$\pm$1.0 days after the CT scan. Overall, there was a moderate correlation with CT muscle CSA (R^2=0.20, p<0.001), suggesting that only 20% of the variance in CT muscle CSA is explained by variance in the ultrasound-muscle thickness indicating other influences are at play as well[74]. With the addition of clinical variables (age, sex, BMI, Charlson Comorbidity Index, and admission type [surgical vs medical]) to quadricep muscle thickness in the model, the correlation was strengthened (R^2=0.61, p<0.001)[74]. Reliability testing revealed a coefficient of variation

of 7% for intra-rater reliability, and 13% for inter-rater reliability[74]. In this study, the relationship between muscularity assessed with ultrasound and a reference technique was weaker than that reported in previous studies as described earlier in this section. The following limitations of this study are noted:

- Multicentre study (multiple investigators and different equipment) and maximal compression technique was used, which may have led to relatively higher inter-rater variability.
- Ultrasound protocol only included lower limbs.
    - Other studies reported stronger associations with the reference method when multiple sites are included presumably because inclusion of more muscle groups may be more reflective of whole-body muscularity[85].
- Did not account for limb length.
    - Accounting for body proportions by multiplying the muscle thickness by the corresponding limb length has shown to improve the correlation between thickness and volume[98].

Despite the limitations detailed above, the VALIDUM study highlighted for the first time that a comparison of bedside assessment of muscularity using ultrasound and a reference method was feasible in the ICU setting, and demonstrated that there was a correlation between the two methods which justified further evaluation of ultrasound. At the time of conceptualisation of research studies for this thesis, it was clear that evaluation of a multi-site ultrasound protocol, using minimal compression and incorporating limb length, compared to a reference method at ICU admission was warranted (Chapter 3).

Identifying individuals with lower-than-normal muscularity with ultrasound

Given the potential utility of ultrasound for providing a quick, prospective assessment of muscularity at the bedside, it is not only important to determine the capability of ultrasound protocols to provide estimates of whole-body muscularity, but also to assess whether those protocols can identify patients with low muscle mass.

A number of studies have investigated the ability of ultrasound to distinguish between individuals with normal and low muscle mass, using a variety of protocols and previously established cut-points using DXA, BIA, MRI, and CT-measured muscularity[74,87,96,99]. When considering ultrasound protocols performed with the subject in the supine position, there are a few studies of interest[74,85,96]. In 96 healthy volunteers, Paris et al demonstrated that the 5-site ultrasound protocol (measuring muscle thickness at the bilateral quadriceps and mid-upper arm) had a strong ability to identify individuals with low lean tissue mass by DXA (area under the curve, AUC = 0.89)[85]. Importantly, utilising the 5-site protocol, the authors developed ultrasound muscle thickness 'cut-points' to categorise individuals into low, moderate, and high risk of low lean tissue mass (for both quadricep and mid-upper arm muscle thicknesses)[85]. However, these thresholds require validation in clinical populations and in relation to clinical and functional outcomes.

Specific to studies within the ICU population, the VALIDUM study (as outlined earlier) explored whether the four-site ultrasound protocol using maximum compression could predict low muscularity determined by CT analysis[74]. This study reported a moderate ability to identify individuals with low CT muscle CSA using the ultrasound protocol (AUC=0.67)[74]. As highlighted earlier, this study had limitations which may have influenced the strength of the findings. Although there are currently limited data (and validated cut-

points) to support using ultrasound to identify individuals with low muscularity using ultrasound, recent studies have shown promise for this purpose.

Limitations of ultrasound for muscle assessment

Despite the potential for ultrasound to be a readily available, non-invasive, and quick tool for muscle assessment, there are a number of limitations. Measurement in morbid obesity is often not possible because excessive adipose tissue can preclude visualisation of the bony landmark on the ultrasound image. Other factors that may impact measurements include the:

- Unknown influence of oedema
- Patient positioning
- Identification of landmarks
- Use of calipers/software for thickness or CSA measurement
- Placement, pressure, and tilt of the ultrasound probe[100]

Nevertheless, if standard operating procedures are developed and adhered to, if appropriate training is provided, and if care is taken while performing the protocol, appropriate inter- and intra-rater reliability for muscle thickness measurements has been reported across various ultrasound protocols, including the four-site protocol in critical illness[89]. As highlighted in multiple review articles, it is imperative that studies using ultrasound for muscle assessment explicitly define their protocols and undertake inter- and intra-rater reliability testing for both landmarking and image acquisition[89].

Bioimpedance technology

Bioimpedance technologies are a widely studied bedside body composition assessment method and have been used in many clinical populations, due to their precision, ease of use, and non-invasive technique[68]. Bioimpedance techniques involve the application of a weak alternating current at one or more frequencies, through electrodes typically attached to, or in contact with, the hands and feet of an individual[68]. The differential flow of the current varies depending on body composition; for example, muscle and blood easily conduct the current whereas bone and fat do not[68]. The voltage detection electrodes detect the drop in voltage as the current passes through the body and the raw impedance data (e.g. resistance, reactance, phase angle) is recorded by the bioimpedance device[68]. Using raw impedance data, in combination with other covariates such as sex, weight, height, bioimpedance technologies can estimate several body compartments that then can be used to estimate fat-free mass (FFM)[101].

There are three types of bioimpedance devices; single-frequency and multi-frequency bioimpedance analysis (BIA) and bioimpedance spectroscopy (BIS)[68]. Each of these uses the impedance data differently to generate whole-body lean tissue mass (eg. FFM) or fluid volumes (e.g., total body water, TBW; extracellular water, ECW; intracellular water, ICW), using underlying assumptions and algorithms[68]. Table 1.6 details the measurement techniques of the three different bioimpedance techniques.

Table 1.6 Description of bioimpedance techniques [68,101,102]

Technique	Details
Single-frequency	• Measures impedance variables at a single frequency (e.g. 50kHz). • Measurement of impedance at only one frequency is theoretically unable to differentiate between ECW and ICW (only TBW), and thus does not provide any ability to identify an individual with overhydration, with the implication being potential to overestimate FFM. • Prediction of compartments based on static relationships between the body compartments from normative data, may not hold true in clinical and obese populations. • Raw data (e.g. phase angle) may have more applicability in clinical populations than body composition estimates which are based on multiple assumptions.
Multi-frequency	• Measures impedance at two or more frequencies including at least one low (typically 5kHz) and multiple higher ones (usually 50, 100, 200, and 500kHz). • Similar to single-frequency devices, the approach estimates FFM using linear regression-derived, population-specific equations, which limits the applicability in the clinical setting and in individuals with obesity where underlying assumptions are violated. • Unlike single-frequency, because it measures at both low and high frequencies, it theoretically allows for the estimation of both ECW and ICW thereby potentially allowing detection of individuals with overhydration. However, in individuals with fluid overload where TBW is higher than in their dry state this will still result in over estimation of FFM (without a correction for overhydration, see below). • Variable accuracy in clinical and obese populations. • Raw data (e.g. phase angle) may have more applicability in clinical populations than body composition estimates which are based on multiple assumptions. • Revised equations correcting for overhydration[103] and adipose tissue[104] have been proposed but not yet validated in many clinical populations.
Bioimpedance spectroscopy (BIS)	• BIS devices use a more physiological approach based on biophysical modelling to predict whole-body volumes. • The electrical current is applied over a range of frequencies from very low (eg, 1 or 5khz) to very high (eg, 1000-1200kHz), measuring impedance data at 50 or more frequencies. • Variable accuracy in clinical and obese populations. • Raw data (e.g. phase angle) may have more applicability in clinical populations than body composition estimates which are based on multiple assumptions. • Revised equations correcting for overhydration[103] and adipose tissue[104] have been proposed but not yet validated in many clinical populations.

The advantages of bioimpedance analysis are well described; it is a portable, safe, low cost, precise (low interobserver variability), quick, and well-tolerated method[68]. However, the method is indirect, using raw data and a number of assumptions, (including normal hydration and adiposity), to estimate FFM. For this reason, the applicability of the type of bioimpedance device in different clinical conditions is an important consideration. Despite this, clinical nutrition societies consider bioimpedance technology an acceptable method for identifying individuals with low muscularity for diagnosis of malnutrition[62,105]. More detail on the generation of FFM values and considerations in critical illness is provided below.

Considerations for use of bioimpedance-derived FFM in critical illness

In general, FFM estimates are considered accurate at the group level in healthy adults when compared with reference methods[102]. However, the bioimpedance approach involves a number of assumptions, including consistent hydration of FFM at 73%, stability of distribution ECW and ICW, and constants for body shape and density[102]. Hence, FFM estimates generated using bioimpedance devices, to-date, have been considered problematic in critically ill patients, given that FFM is derived from a fixed assumption of relationship to TBW which will not hold true in oedema which is common in critical illness[68,106].

Despite these limitations, several alternatives are being explored and show promise for deriving fluid-adjusted estimates of lean tissue[68]. A conceptual model, known as the Chamney model, has been developed from cadaver data and applied to BIS data to assess body composition in dialysis populations, another population that experiences significant fluid shifts[103]. The technique involves an adjustment for excess fluid/overhydration based

on normal hydration of lean and adipose tissue, an important consideration due to the risk of overestimation of FFM due to excess fluid in critical illness[103]. The model uses BIS-derived ECW and ICW, to generate a *normally-hydrated* lean tissue variable. At the time of conceptualisation of the research projects in this thesis, there were no known studies comparing FFM estimates from bioimpedance technology to a reference method in critical illness. This was identified as a significant knowledge gap that needed to be addressed (Chapter 4).

Phase angle

Due to the inherent limitations with FFM estimates using bioimpedance technology, recent clinical investigations have focused on the raw impedance variables (which are independent of weight and height), most commonly 50kHz phase angle[68]. Phase angle is generated from the arctangent ratio of reactance to resistance at 50kHz, and is calculated as the following equation:

$$\textit{Phase angle} = \textit{arctangent (reactance / resistance)} \times 180° / \pi$$

Phase angle is believed to be an indicator of cellular health, with low values reflective of depleted body cell mass[68]. Specific to critical illness, phase angle is emerging as an independent prognostic indicator of clinical outcome[107-112]. In an international, multicentre study of critically ill patients (n=931), day 1 phase angle was lower in patients who subsequently died compared to survivors (4.1° ± 2.0° vs 4.6° ± 1.8°, p=0.001)[112]. The overall day 1 phase angle AUC for 28-day mortality was 0.63 (95% CI 0.58-0.67) and phase angle was independently associated with adjusted 28-day mortality (0.86 95% CI 0.76-0.96, p=0.008)[112]. The underlying link between phase angle and clinical outcome is not

entirely clear, although a relationship between phase angle and muscle health (mass and quality) are thought to exist.

At the time of conceptualisation of the research in this thesis, there was one published multi-centre study in critically ill patients (n=71) comparing phase angle to CT-derived muscularity at ICU admission, finding a weak correlation between the two (adjusted R^2=0.20)[110]. With covariates (age, sex, BMI, Charlson Comorbidity Index, and admission type) added to the model, the relationship was strengthened (adjusted R^2=0.61)[110]. These preliminary findings suggest that phase angle reflects muscularity but that the majority of the variance in muscularity is explained by other factors, including those in the multivariate model (which still left 39% still unexplained)[110].

Multi-centre studies have many benefits in terms of generalisability of the results. However, when multiple investigators and sites are involved, training and standardisation of measurements are critical to ensure repeatable measurements using the bedside body composition method. While the level of precision for single- and multi-frequency bioimpedance devices has been reported as very good in the literature (1-2% variability), the level of accuracy in clinical populations with fluid overload may be more variable[68].

As outlined earlier, the accuracy of bioimpedance measurements may also be impacted by obesity and substantial fluid and electrolyte shifts. Additionally, there are multiple factors that may introduce measurement error or inconsistency in results when performing a bioimpedance measurement within the hospital setting. For example, patient positioning (limbs must be separated and patient resting in the supine position), with minor deviations contributing to 2-3% variability, and 18-43% when skin-to-skin

contact between limbs has been reported[113,114]. The placement of the voltage detection electrodes is also important, with incorrect placement resulting in up to 2% variability[114]. Although not always possible or relevant in the acute setting and particularly in critical illness, additional fluid (e.g. not voiding before a test), caffeine, smoking and strenuous exercise prior to a test may also influence bioimpedance results[68]. Although bioimpedance techniques have been criticised for their limitations, including multiple underlying assumptions which may be violated in illness, as previously outlined, the method is non-invasive, cheap, and easy to use and more research into the applicability of bioimpedance data (including raw data) in highly vulnerable clinical populations is warranted.

Validation of bioimpedance technology to estimate muscularity compared to a reference method in critical illness

As detailed above, at the time of conceptualisation of the research in this thesis, there were no known studies comparing bioimpedance-derived FFM values and phase angle with a reference method for muscle assessment in critical illness. Since this time, there have been a small number of studies[115,116], which along with the findings of the research from this thesis, are discussed in Chapter 4.

Ability of bioimpedance technology to identify patients with low muscularity

Malnutrition, in particular, undernutrition is defined as *"a state resulting from lack of uptake or intake of nutrition leading to altered body composition (decreased fat-free mass and body cell mass) leading to diminished physical and mental function and impaired clinical outcomes of disease"*[105]. In 2015, the European Society of Parenteral and Enteral Nutrition (ESPEN) published a consensus statement on malnutrition diagnosis[105] and in 2019, the Global Leadership Initiative on Malnutrition (GLIM) published a minimum set of

criteria for the diagnosis of malnutrition, including the identification of individuals with low muscularity as a key criterion for diagnosis[62,105]. Specifically, the GLIM diagnostic criteria are based on consensus among experts representing several major global clinical nutrition societies[62]. To diagnose malnutrition, the GLIM criteria require at least one phenotypic criterion (weight loss, low body mass index, low muscle mass) and one aetiologic criterion (reduced intake, altered absorption, acute and/or chronic inflammation), Table 1.7[62].

Table 1.7 GLIM Criteria for the diagnosis of malnutrition. Adapted from Cederholm et al[62]

	Phenotypic Criteria*			**Aetiologic Criteria***	
	Weight loss (%)	Low body mass index (kg/m^2)	Reduced muscle mass	Reduced food intake or assimilation	Inflammation
Moderate Malnutrition	>5% within past 6 months, or>10% beyond 6 months	<20 if <70 years <22 if ≥70 years Asia: <18.5 if <70 years, or <20 if >70 years	Mild to Moderate deficit (per validated body composition methods)	≤50% of energy requirements >1 week, or any reduction for >2 weeks, or any chronic gastrointestinal condition that impacts food absorption	Acute disease/injury or chronic disease-related
Severe Malnutrition	>10% within the past 6 months, or >20% beyond 6 months	<18.5 if <70 years <20 if ≥70 years	Severe deficit (per validated body composition methods)	As above	As above

*A malnutrition diagnosis requires at least 1 phenotypic criterion and 1 aetiologic criterion

Within these diagnostic frameworks, bioimpedance analysis is identified as one of the potential methods to assess muscularity and detect reduced muscle mass[62]. The ESPEN group was the first clinical nutrition society to endorse the following bioimpedance generated sex-specific cut-points to identify those individuals with low muscularity (using FFM index [FFMI] defined as FFM divided by height in metres squared): <15kg/m^2 for women and <17kg/m^2 for men[105]. These cut-points were derived from a cross-sectional

study, including 5635 healthy Swiss volunteers varying in age from 24 to 98 years old[117]. Single frequency BIA was used to measure FFM and cut-points were based on the BMIs of the reference population, with low FFMI values aligned with BMI<20kg/m^2 [117]. FFM derived from single frequency BIA had been validated against DXA in an earlier study, of 343 healthy volunteers between 18 to 94 years old with a BMI ranging from 17 to 34 kg/m^2 [118]. At the time of conceptualisation of the research studies in this thesis, there were no known studies investigating the relationship between low muscularity measured by bioimpedance technology and a reference method in the ICU setting.

Limitations with bioimpedance technology

Contraindications for performing a bioimpedance measurement are few, and include the presence of a pacemaker or implantable mechanical device, unable to access sites for electrode placement (hands and feet), or a limb amputation(s). However, as outlined in earlier sections, although bioimpedance measurements can be performed widely, it is important to consider the multiple factors that might interfere with the accuracy of results. These include: obesity, fluid and electrolyte shifts, patient positioning, electrode placement, fasting status, skin temperature, poor skin integrity at site of electrode placement, and proximity to medical devices[68]. It is important that researchers and clinicians are aware and where possible, eliminate or limit these impacting factors, or exclude patients who are not appropriate for testing (e.g. obesity, marked oedema).

Consideration for bioimpedance data interpretation

Additionally, with certain devices such as BIS, raw data downloaded from the device can and should be used to determine the validity of the measurement (e.g., FFM values which

may be impacted by extreme fluid overload). Examples include accepting the measurement if the:

- Cole plot follows a half semi-circular pattern, standard error of estimation (SEE) fits to the curve below 1.0;
- Intracellular resistivity (Ri) is *greater* than extracellular resistivity (Re); and
- Whole body FFM values are within physiological limits (e.g. none of the water or FFM values larger than body weight, ECW as a percentage of TBW ratio, ECW to ICW ratio – with fluid overload indicated if the ratio approaches 1)[119].

Arm anthropometry

Arm anthropometry is a relatively inexpensive, rapid, portable, non-invasive method that has been used as a proxy method for body composition analysis in many clinical settings where there is absence of access to other body composition methods[69]. The method involves assessing the dimensions of the upper arm including: upper arm length, mid-upper arm circumference (MAC), and triceps skinfolds (TSF). These measurements can also be used to derive a surrogate assessment of lean body mass, such as mid-arm muscle circumference (MAMC) using the formula by Heymsfield et al., and mid-arm muscle area (MAMA)[120]. These equations are derived from non-obese, healthy volunteers[120]. Large datasets such as those derived from nationwide surveys, such as the National Health and Nutrition Examination Survey (NHANES) from the United States of America, provide valuable anthropometric reference data for adults and children (which is further stratified by gender and age)[121]. Using these reference data, clinicians and investigators have classified individuals as having low muscularity by a measurement being attributed to a low percentile (e.g. <15th percentile)[66].

In critical illness, arm anthropometry has been used in a small number of studies to evaluate the relationship between baseline nutrition status and clinical outcomes, with mixed findings[122-125]. In the largest study, 1363 patients were enrolled from 31 different ICUs throughout Australia and New Zealand, aiming to assess the ability of baseline physical and anthropometrical data (including TSF, MAC, and MAMC) to predict hospital mortality[123]. When observing the arm anthropometry data, TSF was not significantly associated with hospital mortality (Odds Ratio [OR] 1.01; 95% CI, 0.99-1.02, p=0.32)[123]. For univariate analysis both MAC and MAMC demonstrated acceptable ability to predict hospital mortality (OR 0.97; 95% CI, 0.94-0.99, p=0.01 and OR 0.95; 95% CI, 0.93-0.98, p<0.001, respectively). However, after adjusting for confounders, only MAMC remained in the final multivariate model[123]. Although there appeared to be significant training and support provided by an experienced investigator in this multi-centre study, neither intra- nor inter-rater reliability testing of the protocol were reported[123].

Other studies have taken longitudinal measurements of MAC during ICU admission[94,126]. In the study by Campbell et al., nine patients with multiorgan failure were studied, and muscle thickness (via ultrasound) and MAC were measured every 1-4 days early in the ICU admission[94]. Using ultrasound, all patients showed a significant, consistent decrease in muscle thickness over time[11]. In contrast, the arm circumference measurements showed no consistent pattern of change[94]. Although arm anthropometry can be easily applied at the bedside, these findings indicate that oedema may influence measurements in the ICU setting resulting in the overestimation of muscularity (and risk of failing to identify patients with muscle depletion) and potentially over-predict loss of muscle mass when in fact loss is due to resolution of oedema (not true loss of muscle).

Validation of arm anthropometry as a surrogate measure of muscularity in critical illness

At the time of conceptualisation of the research studies in this thesis, to the candidate's knowledge no study had performed a comparison between arm anthropometry measures with a reference method for measuring muscularity in critically ill patients. This is an important knowledge gap that is addressed in Chapter 4.

Limitations of arm anthropometry

There are a number of limitations of arm anthropometry that need to be considered for the utility of the method in critical illness, including: the unknown influence of oedema and obesity (particularly for MAC), the potential for errors and consistency in patient positioning, identifying bony landmarks for the points of measurement, and unclear reference standards for defining individuals with low muscularity[69]. Furthermore, the technique assumes a linear relationship between a single upper limb measurement and whole-body muscularity which may not always hold true.

Subjective physical assessment of muscularity

A number of malnutrition diagnostic tools recommend a subjective physical assessment of muscularity when other body composition methods are unavailable or feasible to undertake for quantitative muscle mass assessment[62-64,105]. For example, the subjective global assessment (SGA) recommends the following anatomical areas are assessed subjectively for muscle wasting: temple, clavicle, shoulder, scapula/ribs, quadriceps, calf, knee, and interosseous muscle (assessed by pressing the thumb and forefinger together)[63]. Using this method, the clinician can make a subjective assessment of muscle wasting based on the observed findings – no sign, mild-moderate or severe muscle

wasting[63]. However, assessing muscularity using this method can be challenging in the ICU setting with patients often bedridden and attached to medical equipment limiting the anatomical sites for assessments. Furthermore, obesity and oedema may mask underlying muscle depletion. Evaluation against an objective reference method for identifying muscle wasting is critical to determining the utility of this method to identify patients with low muscle mass. The ability for the method to detect and quantify change over time and to assess the response to an intervention aimed to attenuate muscle loss is also unknown.

Comparison of subjective physical assessment with a reference method to identify patients with low muscularity in critical illness

At the time of conceptualisation of the research studies in this thesis, there was one known study which compared SGA-determined nutritional status at ICU admission with CT-measured muscularity (using the full SGA not just the muscle wasting component)[127]. The study enrolled 56 patients, who were admitted to an ICU with respiratory failure, and who also had a CT Scan at the L3 area performed for clinical purposes during hospitalisation[127]. For the 36 patients who had a SGA and CT scan performed within 7 days of each other, misclassifications (i.e. ranked as normally nourished on SGA but had low CT-measured muscularity) were observed in 50% of the patients with low CT muscularity[127]. Where the SGA was performed within 3 days of the CT scan (n=25), similarly, 63% patients who had low CT muscularity were misclassified as normally nourished on the SGA[127]. Misclassified individuals were predominantly male, of a minority ethnic group, and/or overweight and obese. This study had limitations, including the time period between performing the SGA and the CT scans[127]. Critically ill patients lose muscle rapidly in the ICU, and ideally the time difference between comparator muscle assessment methods should be limited, with a 48-hour cut-off between techniques suggested to be

appropriate[74]. Furthermore, in this study the whole SGA tool (not just the muscle assessment) was used to make the overarching nutrition assessment. This tool includes a number of other components, including weight, height, weight and diet history, gastrointestinal systems, and nutrition-related functional status[63]. Collecting this history is almost always impossible to obtain early in the ICU admission when patients are intubated and sedated unless a proxy is able to reliably provide a history[61]. Therefore, the overarching nutrition assessment by SGA for comparison to CT-measured muscularity may have been influenced by other components. Hence, the ability of a subjective physical assessment to identify patients with low muscularity at ICU admission compared to a reference method remains unknown. Chapter 4 aims to address this knowledge gap.

1.4.1.3 Body composition variation

The third body composition research area is the measurement of variation in body composition compartments. This kind of analysis is done to understand the changes that occur in particular clinical situations or disease states, to investigate variables influencing changes, and/or to monitor a response to an intervention (e.g. high protein diet for muscle maintenance)[66]. When using any body composition technique, it is important to consider whether the method has appropriate precision to detect clinically significant changes in the body composition compartment of interest[68]. The next section outlines considerations with methods to longitudinally measure muscularity in the ICU setting.

Considerations of using body composition techniques to measure changes in muscularity in critical illness

There are a number of studies which longitudinally measured changes in both muscle mass and quality in critical illness. The most common assessment methods that have been used are CT image analysis and ultrasound (as outlined in Section 1.3.2). However, as previously mentioned, there are many factors which may impact the precision of body composition techniques in critical illness, and particularly when observing changes over time, which should be considered (most notably significant fluid shifts).

As described earlier, the transport of critically ill patients to specialised machinery for body composition analysis is not often feasible nor appropriate. CT scans can be utilised, and are considered a reference technique but are usually only available when performed for clinical purposes, which is often on an *ad hoc* basis. Due to these feasibility limitations with performing reference body composition techniques at pre-determined points in critically ill patients, the comparison between two different methods for muscle assessment over time is challenging, and to our knowledge has not been done before. Therefore, other ways to determine the ability of techniques to detect changes in critical illness, should be examined. Chapter 6 explores the short-term precision of CT image analysis to detect changes in skeletal muscle CSA and quality using information that is available as part of routine care.

1.5 Nutrition assessment and therapy in critical illness

In critical illness, patients are often unable to consume any or enough nutrition orally due to various reasons including: mechanical ventilation, increased metabolic requirements, poor appetite, altered conscious state, gastrointestinal intolerance, and swallowing difficulties[60]. For these reasons, as well as the potential for pre-admission disease processes impacting negatively on nutritional status, clinical guidelines highlight that all patients admitted to the ICU for >48 hours are at high nutrition risk, and are likely to require nutrition support[18]. The provision of early enteral nutrition to critically ill patients is an accepted standard of care which aims to; minimise negative energy and protein balance, avoid starvation, maintain tissue function, preserve skeletal muscle mass and support recovery in the post-ICU period[24].

To enable an appropriate nutrition intervention, it is imperative that a trained nutrition professional (e.g. a dietitian) undertakes a comprehensive nutrition assessment. A nutrition assessment in general hospitalised patients should include the components highlighted in Table 1.8[24].

Table 1.8 Components of a nutritional assessment in general hospitalised patients[24,62]

Component	Details
Measurement of nutrient balance	• Weight loss, appetite, dietary intake, fluid balance, gastrointestinal symptoms, nutrient losses, and medical and drug history should all be assessed • Detailed diet history is a critical measure
Measurement of body composition	• Weight, height, and body mass index are basic measures that should always be obtained • Assessment of muscularity and in particular, identification of muscle depletion which is highly associated with malnutrition, is included as key criterion in recent assessment tools
Measurement of disease severity or inflammation	• Involves assessing clinical history and current state, bedside vital signs (e.g. temperature, biochemistry)
Measurement of function	• Physical dysfunction is associated with malnutrition and should be considered where possible for both defining initial status and for continual monitoring (e.g. hand grip strength)

1.5.1 Nutrition assessment in critical illness

As highlighted in previous sections, early in critical illness when patients are often sedated and bed-ridden, undertaking a nutritional assessment including components as highlighted in Table 1.8 above, is challenging. The development and evaluation of techniques to objectively assess components related to malnutrition, such as muscle mass, may facilitate early identification of individuals with malnutrition who may benefit from early and more intensive nutritional therapy especially when patient-elicited information is not available.

1.5.2 Nutrition therapy in critical illness

The nutritional needs of a critically ill patient will depend on pre-admission nutritional status, phase of critical illness (i.e. early, late or recovery), as well as a range of other factors which will alter metabolic requirements (e.g. organ support, infection, temperature, wounds, injuries)[18]. International ICU nutrition guidelines now recommend that energy delivery is based on measured energy expenditure by indirect calorimetry, due to the errors associated with energy estimations when predictive equations are used[17,18,128]. Despite these recommendations, in a recent survey of 63 Australian and New Zealand ICU dietitians, only 21% of respondents reported they had access to an indirect calorimetry device[129]. Limitations to using indirect calorimetry are believed to include the cost of the indirect calorimetry device, reproducibility of results, time for set-up and measurement, and lack of expertise in the application of the equipment and funding for staff time[129,130]. Furthermore, the method is not appropriate for all critically ill patients (e.g. receiving extracorporeal membrane, high pressure support and/or oxygen requirements). As such, for now, nutrition delivery in critical illness is still largely driven by predictive weight-based equations (with different countries and centres using different equations) which may result in under- or over-feeding[128] and may have a marked cumulative impact in those patients with a longer ICU admissions. The development of a new generation indirect calorimeters, which are much quicker and easier to use and can be applied in a broader range of patients, may result in this technique being more widely used in clinical practice[130].

While the delivery of early enteral nutrition is an accepted international standard of care, it is well documented that ICU patients receive less nutrition than prescribed and recommended in international clinical guidelines[131]. This is primarily due to delayed

initiation, gastrointestinal intolerance, and fasting for procedures[131]. There is also a growing body of evidence demonstrating that nutrition delivery remains compromised when patients are discharged to a ward, compounded by the early removal of nasogastric tubes, poor appetite, taste changes, and swallowing difficulties[132]. As a result, critically ill patients are at high risk of significant cumulative deficits in energy and protein delivery throughout the whole hospital admission, which may negatively impact muscle mass.

1.5.2.1 Nutrition delivery and association with skeletal muscle mass changes in critically illness

As highlighted in section 1.2.2, in health skeletal muscle is highly responsive to nutrition interventions. The belief is that nutrition therapy may help attenuate skeletal muscle loss in critical illness[18]. At the time this doctoral research commenced, the literature investigating nutrition delivery and skeletal muscle mass changes had not been reviewed systematically. Chapter 5 addresses this gap in the literature.

1.6 Summary

This literature review has highlighted that skeletal muscle mass and quality at ICU admission, as well as deterioration in both components during the first few weeks of critical illness, is associated with poor recovery. Being able to objectively assess muscularity at the bedside in critical illness, using an accurate, quick, and reliable method may help to identify patients at high risk of prolonged rehabilitation and evaluate strategies and therapies aimed at attenuating the losses that occur. There are currently limited studies evaluating the ability for bedside methods to objectively measure muscularity (and identify low muscle stores) when compared to a reference method in critical illness

Furthermore, the investigation of strategies aimed at attenuating muscle loss in critical illness, such as nutrition therapy, is required. Additionally, patients who stay the longest in the ICU are at the highest risk of prolonged recovery. Most studies have observed changes in skeletal muscle mass and quality over the first weeks of critical illness. There are limited data using a reference method that quantifies muscle mass and quality changes according to week of critical illness.

1.7 Thesis aims and outline

The overall aims of this thesis were to compare muscularity assessed by easily applied bedside body composition methods with a reference technique in critically ill patients at ICU admission and to investigate changes in muscle mass and quality according to week of critical illness and the association with energy and protein delivery.

1.7.1 Objectives

1. To compare muscularity assessed by a multi-site bedside ultrasound protocol and CT image analysis at ICU admission (Chapter 3)
2. To investigate the ability of the ultrasound protocol to identify patients with low CT-measured muscularity (Chapter 3)
3. To compare muscularity assessed by bioimpedance technology, arm anthropometry, and subjective physical assessment and CT analysis at ICU admission (Chapter 4)
4. To investigate the ability of bioimpedance technology, arm anthropometry, and subjective physical assessment to identify patients with low CT-measured muscularity (Chapter 4)
5. To investigate the association between energy and protein delivery and skeletal muscle changes in critical illness (Chapter 5 and 6)
6. To explore the precision of CT image analysis to detect changes in muscle mass and quality in critical illness (Chapter 6)
7. To explore changes in CT-measured muscle mass and quality according to week of critical illness (Chapter 6)

1.7.2 Thesis structure

The aims and objectives of the doctoral thesis are addressed through the results of a pilot prospective observational cross-sectional study (ICU-Muscle), systematic literature review, and a retrospective observational study.

Chapter 2 provides a detailed description of the methods used in the original research studies of this thesis (ICU-Muscle and retrospective study).

Chapter 3 evaluates the relationship between muscularity assessed by a multi-site bedside ultrasound protocol (using minimal compression) and CT image analysis at ICU admission. It also reports detailed reliability testing and evaluates the ability of the ultrasound protocol to identify patients with low CT-measured muscularity.

Chapter 4 explores the relationship between muscularity assessed by bioimpedance technology, arm anthropometry, and subjective physical assessment and CT analysis at ICU admission. It also explores the ability of each method to detect patients with low CT-measured muscularity.

Chapter 5 reports a systematic review which synthesises the existing literature investigating the relationship between energy and protein delivery on skeletal muscle mass changes in critical illness and identifies gaps in the literature.

Chapter 6 describes changes in CT-measured muscle mass and quality across different weeks of critical illness and the association of energy and protein delivery on these

changes (using data obtained from the medical records going back 10 years). This study also explores the precision of CT image analysis to detect changes in muscle mass and quality in ICU patients.

Chapter 7 draws the results of chapters three to six together and discusses the clinical implications and potential future directions for research in this field.

A summary of the thesis objectives and corresponding Chapter and study are displayed in Table 1.9 below.

Table 1.9 Thesis objectives and corresponding studies, chapters and aims

Thesis Objective	**Study**	**Methods Chapter Section**	**Study Aims**	**Results Chapter**
1. To compare muscularity assessed by a multi-site bedside ultrasound protocol and CT image analysis at ICU admission	Pilot prospective observational cross-sectional study (ICU-Muscle)	2.2	2.2.2 (1) 2.2.2 (2)	3
2. To investigate the ability of the ultrasound protocol to identify patients with low CT-measured muscularity	Pilot prospective observational cross-sectional study (ICU-Muscle)	2.2	2.2.2 (3)	3
3. To compare muscularity assessed by bioimpedance technology, arm anthropometry, and subjective physical assessment and CT analysis at ICU admission	Pilot prospective observational cross-sectional study (ICU-Muscle)	2.2	2.2.2 (4)	4
4. To investigate the ability of bioimpedance technology, arm anthropometry, and subjective physical assessment to identify patients with low CT-measured muscularity	Pilot prospective observational cross-sectional study (ICU-Muscle)	2.2	2.2.2 (5) 2.2.2 (6)	4
5. To investigate the association between energy and protein delivery and skeletal muscle changes in critical illness	Systematic literature review and retrospective observational study	2.3 + Chapter 5 methods	2.3.2 (2) + Chapter 5	5, 6
6. To explore the precision of CT image analysis to detect changes in muscle mass and quality in critical illness	Retrospective observational study	2.3	2.3.2 (3)	6
7. To explore changes in CT-measured muscle mass and quality according to week of critical illness	Retrospective observational study	2.3	2.3.2 (1)	6

Chapter 2

Study Methodologies

2.1 Introduction

This chapter outlines and provides justification for the methods employed in the two distinct original research studies conducted in this thesis (Chapter 3, 4, and 6). These studies were specifically developed and conducted to answer the aims and objectives of the candidate's doctoral research. A description and breakdown in the research tasks undertaken by the candidate is provided in Appendix 2. The description of the methods for the systematic review, which aimed to investigate the association between energy and protein delivery and skeletal muscle mass changes are encompassed within Chapter 5.

2.2 The evaluation of bedside methods to measure muscularity in critically ill patients: a prospective observational study (ICU-Muscle Study)

2.2.1 Study design

The ICU-Muscle study was a prospective cross-sectional single-centre observational study which was conducted at The Alfred Hospital in Melbourne, Australia. It was designed to address thesis objectives 1-4 (see Table 1.9).

2.2.2 Study aims and hypotheses

The primary aim of this study was to:

1. Compare ultrasound-derived muscle thickness at five different anatomical landmarks, with muscularity assessed by a reference method (CT image analysis at the third lumbar [L3] area).

Secondary aims were to:

2. Evaluate if combining ultrasound-derived muscle thickness at different anatomical landmarks and readily available patient information could strengthen the correlation with CT muscle CSA.
3. Assess the ability of the best-performing ultrasound model to accurately classify patients with low CT muscle CSA.
4. To determine the association between bedside measures of muscle mass (BIS and arm anthropometry) and CT muscle CSA at ICU admission.

5. To assess the agreement between muscularity status (low or normal) assessed by BIS, arm anthropometry, and subjective physical evaluation of muscle stores and CT image analysis.
6. To evaluate how BIS-derived phase angle relates to CT muscle CSA and density.

The key hypothesis for this study was that there would be a strong linear association (r > 0.8) between ultrasound muscle thicknesses (cm) and CT muscle CSA (cm^2) at ICU admission (relates to aim 1).

2.2.3 Study setting

The study was conducted at The Alfred Hospital in Melbourne, Australia. The Alfred is an adult tertiary teaching hospital with a mixed ICU caseload (intensive care and high dependency beds). At the time this study was performed (2017 – 2019, before the COVID-19 pandemic) there were 45 available beds in the ICU. The Alfred provides state-wide services for adult trauma, heart and lung transplantation, artificial heart technology, extra-corporeal membrane oxygenation (ECMO), burns, HIV, bone marrow transplant and hyperbaric medicine. It is also a referral centre, taking patients from other metropolitan and regional general hospitals and has a casemix comprising both medical and surgical patients. As stated in The Alfred's 2018-2019 annual report, there were 2,842 inpatient episodes involving an ICU stay, with 60 per cent of these patients coming from outside the local catchment area, which is attributable to the state-wide services that care for the most acute patients[133].

2.2.4 Study population

From January 23rd 2017 to 24th March 2019, participants were screened for eligibility from daily ICU ward lists on days when a trained researcher (candidate KL or Dr Jessica Wang, ICU fellow, JW) was available. Patients who met all the inclusion and none of the exclusion criteria, were eligible for enrolment. *Note*: There were no known major changes to patient casemix or standard ICU management protocols during the recruitment period (pre-COVID).

2.2.5 Patient eligibility

Inclusion criteria

Patients admitted to the ICU who met the following:

- ≥ 18 years old
- Had a CT scan including the L3 vertebra performed for clinical reasons ≤24 hours before or ≤72 hours after ICU admission

Exclusion criteria

- Death imminent or deemed highly likely in the next 96 hours
- Known to be pregnant
- BMI > 40kg/m^2 (outside range of previously tested protocol)[94]
- CT scan performed >48 hours at time of screening
- CT scan unanalysable for muscle assessment (e.g. muscle borders indistinguishable, see 2.2.7.1)
- Not possible to image two or more muscle groups (including at least one thigh) via ultrasound (e.g. due to trauma, burns, wounds)

- Treating clinician did not believe participation in the study to be in the best interest of the patient
- Already enrolled in multiple other research projects (The Alfred ICU has a strong research culture, with many research projects being conducted at the same time. To reduce the burden on patients and their families, enrolment in research studies is typically limited to a maximum of two projects).

2.2.6 Patient recruitment

The research team screened the daily ICU list and identified patients who met the inclusion criteria and none of the exclusion criteria. To limit selection bias, screening occurred sequentially and on pre-determined days when an investigator from the research team was available. The research team then discussed potential enrolment in the study with the bedside nurse, treating ICU doctor(s), and ICU research nurses to ensure that the study would not interfere with pending procedures/investigations, other research studies and/or that the patient/family were likely to be open to discussing participation in the study. If all parties were supportive, the research team then approached the patient and/or medical treatment decision maker (i.e. next of kin) to provide a study overview, and if agreeable, the research team obtained informed written consent. As required by The Alfred Ethics Committee, if the medical treatment decision maker initially provided consent, the research team monitored the patient closely and where appropriate, obtained consent from the patient prior to hospital discharge (Note: there were 23 patients who were subsequently able to provide consent after initial consent was provided by the medical treatment decision maker).

2.2.7 Study procedures

After study enrolment, the research team performed the bedside protocol within 48 hours of the CT scan (all bedside measurements were performed in the same session). All study-based data were recorded using a paper case report form (CRF). At a later date, data were transcribed from the CRF into a password-protected excel spreadsheet, and ultrasound and CT images were analysed.

2.2.7.1 CT image analysis

At the time of assessing eligibility, the research team, with assistance from consultant radiologist A/Prof Gerard Goh (consultant radiologist, The Alfred), determined whether the CT scan of a potential study patient was appropriate for the assessment of skeletal muscle mass at the L3 area. Patients were excluded from enrolment if the scan was considered to be non-analysable for muscle assessment (e.g., if the muscle borders were indistinguishable, there was interference of artifact and/or if a muscle group(s) were cut off a scan due to positioning).

Analysis of the CT images was performed using specialised software, sliceOmatic 5.0 (Tomovision, Montreal, Canada). The slice for analysis at the L3 level was identified by scrolling through the axial slices to locate the 12th thoracic vertebrae (T12), with the final rib attachment being the defining characteristic. Once T12 was identified, scrolling continued, moving inferiorly and as the transverse processes disappeared and then re-appeared, the next vertebra was the first lumbar vertebra. This process of scrolling inferiorly continued until L3 was identified. Once L3 was located, there were a few slices

with L3 imaged (depending on the slice thickness). The slice where both transverse processes (left and right) and some marrow were visible was chosen for analysis (see Figure 2.1).

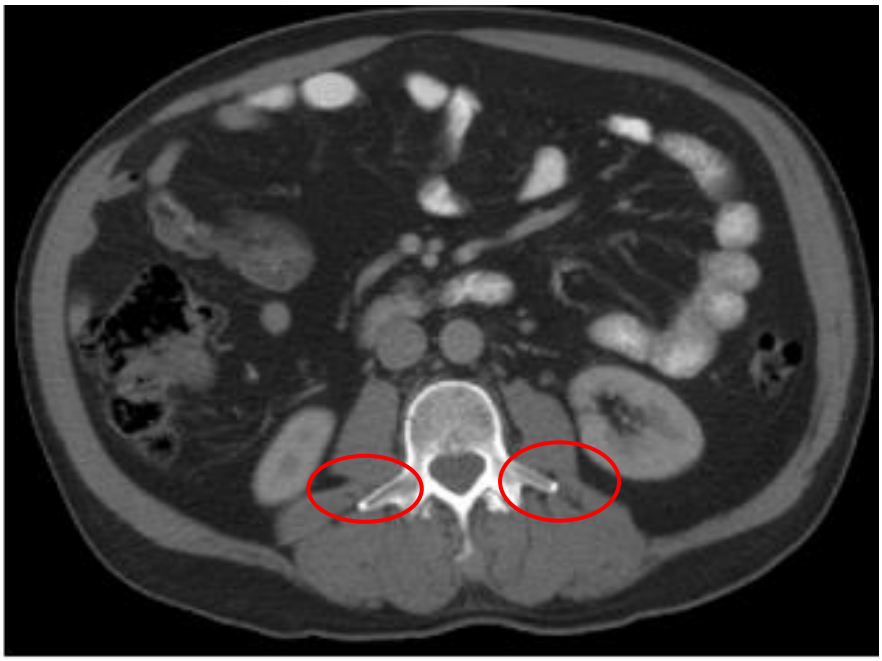


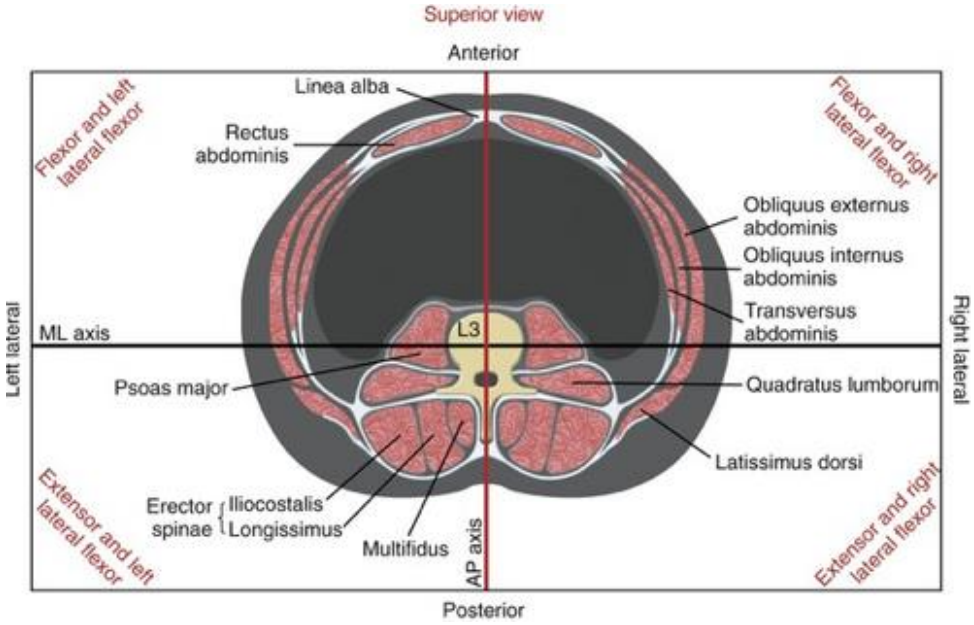
Figure 2.1 The right and left transverse processes of L3 identified on the CT image*

Quantification of skeletal muscle CSA

Using the sliceOmatic software, skeletal muscle boundaries were recognised based on Hounsfield units (-29 to +150 for muscle)[134]. Skeletal muscle CSA (cm^2) was then automatically computed by summing the skeletal muscle tissue pixels and multiplying by the surface area of each pixel. Figure 2.2 displays the muscles measured at L3 area, including the: erector spinae, psoas muscles, obliques and transversus abdominus, and rectus abdominus (A), and an example of a CT slice analysed for muscle assessment using the sliceOmatic software (B).

* *Image taken from study ICU-Muscle study patient*

A.



B.

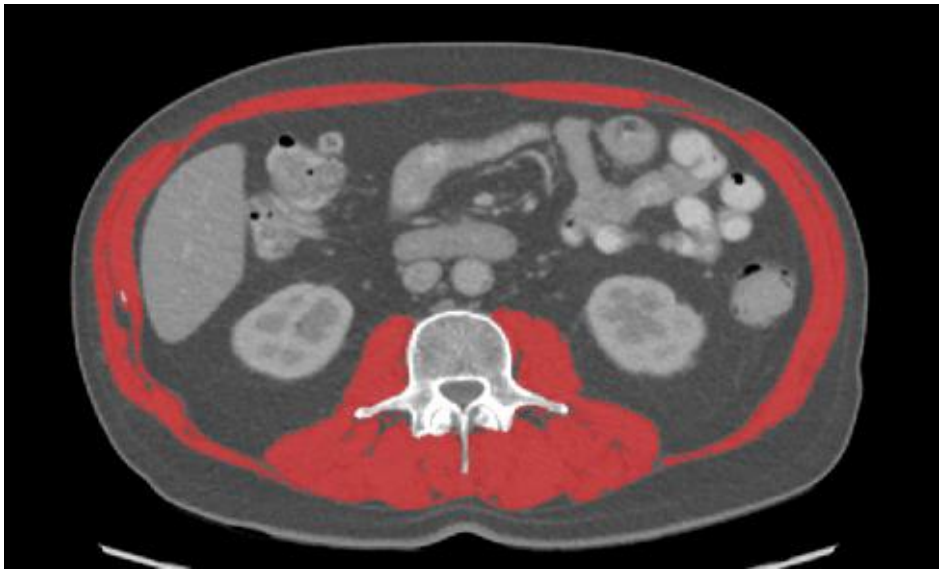


Figure 2.2 Quantification of skeletal muscles at the third lumbar (L3) area;

A. Individual muscle groups at L3*

B. CT slice analysed by the sliceOmatic software, with skeletal muscle highlighted in red†

* *Reprinted from Kinesiology of the Musculoskeletal System, 3rd Edition. Neumann, D. Chapter 10 Axial Skeleton: Muscle and Joint Interactions, p391. Copyright (2017), with permission from Elsevier.*

† *Image taken from ICU-MUSCLE study participant*

Reliability testing

Intra-rater reliability was performed by having candidate, KL, re-landmark and re-analyse ten randomly chosen scans at least six months after initial analysis. Inter-rater reliability occurred by having a second trained investigator (Ms. Lisa Murnane, Dietitian at Alfred Health and PhD Candidate) landmark and analyse ten randomly chosen scans that had been analysed by KL. The plan was for a third person to be consulted to discuss any significant discrepancies in measurements. However, all measurements were similar (coefficient of variation <5%) so a third assessment was not required.

Identification of patients with low muscularity

Muscularity status (normal or low) was determined using cut-points derived from a study carried out in a general ICU population where low CT muscle CSA was associated with mortality (CSA <170cm^2 males and CSA <110cm^2 females)[7]. These cut-points were chosen as they were developed in a population most reflective of the ICU-Muscle patient cohort (Caucasian and admitted to ICU with a range of clinical conditions) and in the absence of any height-adjusted cut-points (cm/m^2) in this population which also had predictive validity.

2.2.7.2 Ultrasound

Trained investigators on the research team (KL or JW) performed the once-off ultrasound evaluation of muscularity as soon as possible after patient enrolment (and within 48 hours of the CT scan). The anatomical sites chosen to compare with CT muscle CSA included muscle thickness of the right mid-upper arm and forearm (left side if right not available),

abdomen, and bilateral thighs (details below). Where possible, rectus femoris CSA was also measured at the two-thirds point on both thighs[89]. These sites were chosen because they are readily accessible while the patient is in a supine position, and the measurement protocols at the upper and lower limbs have been reported as reliable in the ICU setting[74,89,135], and associated with whole-body muscularity in healthy volunteers[85,94,136].

Image acquisition

A portable B-mode ultrasound device (Philips Sparq, Philips Ultrasound, Bothell, WA, USA) with a multi-frequency linear array transducer (4-12 MHz, 5cm width) was used, Figure 2.3. Standard settings for gain and frequency were maintained. Patients were supine with the head of the bed at approximately 30 degrees (usual positioning in The Alfred ICU). A generous amount of water-soluble transmission gel was applied to the transducer. Using minimal compression (just touching the skin), the transducer was held perpendicular to the skin and the depth adjusted so that the relevant bone was visible in the image (e.g. femur with quadriceps muscle) or the inner muscle fascia layers for the abdomen. Three still images were taken at each landmark.

Figure 2.3 Philips multi-frequency linear array transducer*

* *Photo taken for presentation in this thesis (K.Lambell)*

Landmarks

Mid-upper arm

Measurements were taken on the right arm (as per previously published protocols) [94,135]. If it was not possible to access the right arm (e.g., due to trauma), then the left side was used. Where possible, the patient's dominant arm was recorded (reported by the patient or family), to later determine whether this impacted the correlations. With the elbow flexed to 90 degrees, a point was marked on the skin at the tip of the acromion and the tip of the olecranon. If the arm could not be flexed, a measurement was not performed at this site. The midway point between these two measurements was then marked with a pen, Figure 2.4.

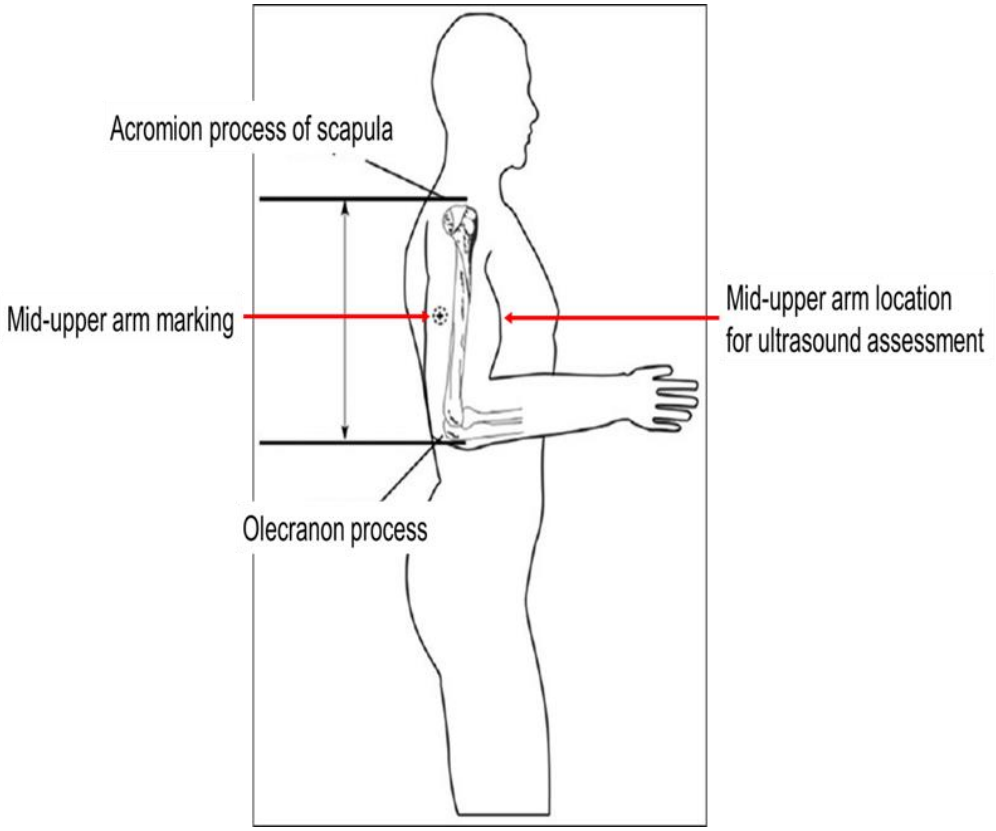


Figure 2.4 Upper arm landmarks and midway point for imaging*

* *Figure adapted from the National Health and Nutrition Examination Survey (NHANES) Anthropometry Manual, 2007. www.cdc.gov/nchs/data/nhanes/nhanes_07_08/manual_an.pdf. Accessed 20th April 2021*

A measuring tape was then placed around this mark (around the circumference of the mid-upper arm), and a point was marked at over the centre point over the bicep, Figure 2.4 and 2.5.

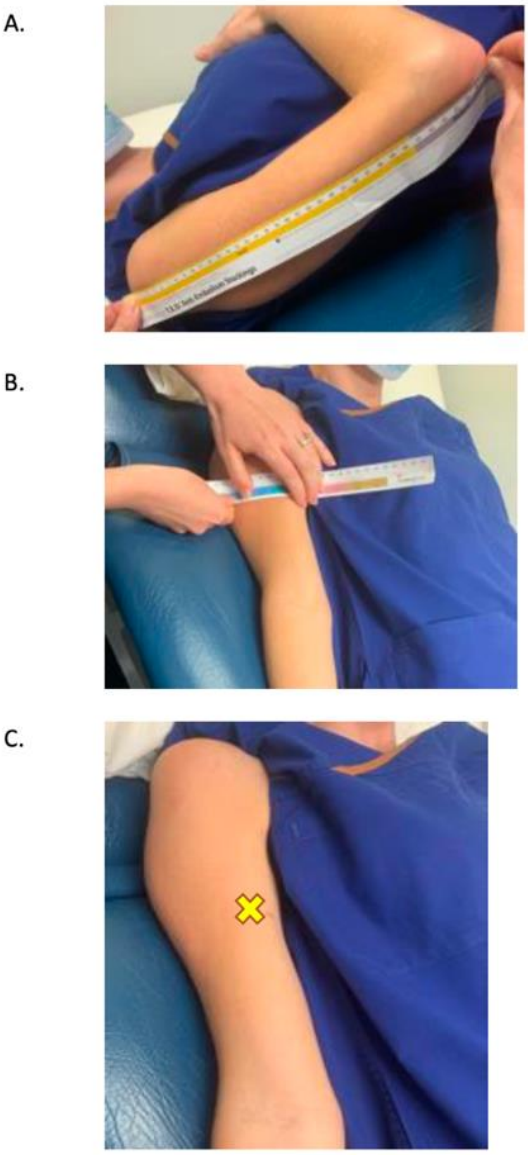


Figure 2.5 Pictures demonstrating the mid-upper arm measurement, A. measurement from acromion process to olecranon process, B. measuring tape tracking from the mid-point across to the centre point over the bicep, C. Yellow cross indicating point for ultrasound measurement*.

* *Photos of a volunteer to demonstrate location of mid-upper arm landmark (K.Lambell). Note: a paper tape measure was used for the photos. A flexible non-stretch tape was used for the landmarking for the ICU Muscle bedside protocol.*

With the elbow extended and the forearm supinated and resting on the bed, the thickness of the flexor compartment was measured at this midway point, over the biceps, between the subcutaneous adipose tissue-muscle interface and the muscle-bone interface of the humerus. Both the biceps brachia and the coracobrachialis were included in the muscle thickness measurement (see example ultrasound image in Figure 2.8).

Forearm

The right arm was used. If it was not possible to image the right arm, then the left was used. A point was marked 30% proximal between the ulnar styloid and the head of the radius. With the hand and forearm supinated, a tape measure was then used to trace around so the point for imaging was marked in the middle of the forearm, Figure 2.6.

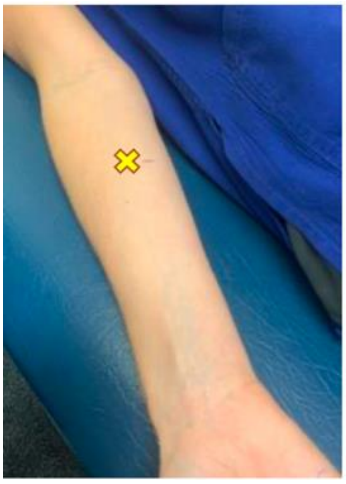


Figure 2.6 Location of forearm landmark (indicated by yellow cross)*

* *Photos of a volunteer to demonstrate location of forearm landmark (K.Lambell)*

With the hand remaining supinated and forearm relaxed on the bed, the ultrasound image was taken. Ulna muscle thickness was measured as the distance between the subcutaneous adipose tissue-muscle interface and muscle-bone interface of the ulna (see example image in Figure 2.8)[136] .

Abdomen (abdominus rectus)

A mark was made 3 cm to the right of the umbilicus (as observed by the researcher looking at the patient's umbilicus). The probe was positioned so that the abdominus rectus muscle could be visualised parallel to the screen and muscle thickness was measured at this point (see example image in Figure 2.8)[83].

Thigh (rectus femoris and vastus intermedialis)

With the patient lying supine, feet were positioned approximately 15cm apart with the quadriceps running in a straight line with the lower leg and great toe facing the ceiling. A towel was placed on the side of each foot to avoid rotation. With knees extended and relaxed, two landmarks on each quadriceps were identified and marked on the anterior surface from 1) the midpoint between the anterior superior iliac spine (ASIS) and the upper pole of the patella and 2) the border of the lower third and upper two-thirds between the anterior superior iliac spine and the upper pole of the patella, see Figure 2.7.

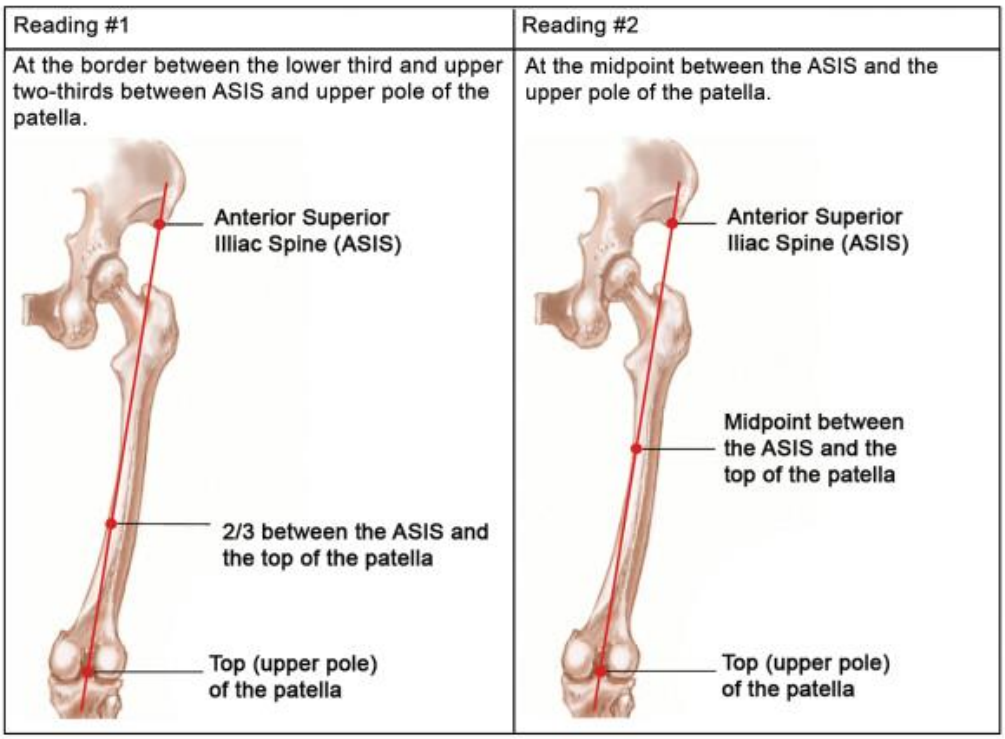


Figure 2.7 Landmarks for ultrasound measurement at two points on the thighs; two-thirds (Reading #1) and midpoint (Reading #2) distance from ASIS to the patella*

Muscle thickness of the thighs was measured at both points on both legs, with the image including both the rectus femoris and vastus intermedialis (see example images in Figure 2.8). Rectus femoris CSA was measured (when visible) at the two-thirds points on both legs[89]. If the above positioning was not possible and both thighs inaccessible, the patient was excluded from participating in the study (as outlined in 2.2.5). The rationale for this was that the quadriceps are the largest muscle group in the body and at least one thigh should be included to test the true ability of the protocol to provide an estimate of whole-body muscularity.

* *Tillquist, M. et al (2014). Bedside ultrasound is a practical and reliable measurement tool for assessing quadriceps muscle layer thickness. JPEN, 38(7), 886-890. Image reproduced with permission, Wiley Materials (copyright)*

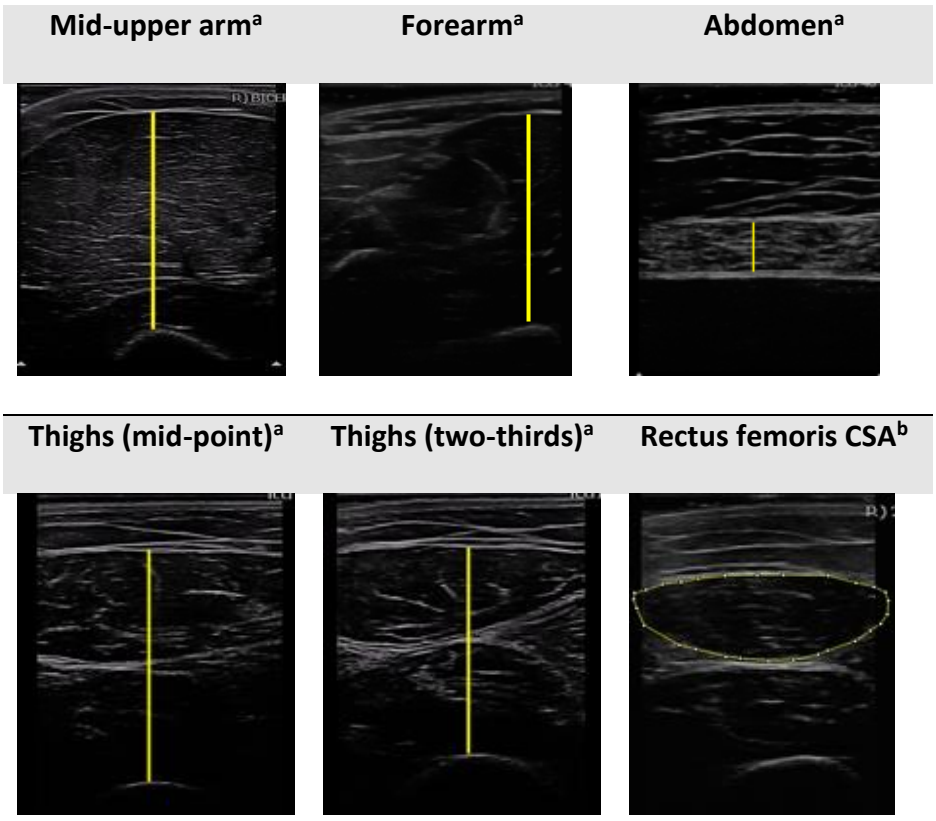


Figure 2.8 Ultrasound images at each anatomical site*. CSA, Cross-sectional area

[a]Vertical yellow line indicating muscle thickness (cm) from superficial fat-muscle interface to the relevant bone or inner muscle fascia layers for the abdomen.

[b]Circular yellow line indicates rectus femoris CSA (cm^2) measured at the two-thirds point on the thighs.

Measurement of muscle thickness and rectus femoris CSA

Measurement of muscle thickness or muscle CSA can either be performed at the bedside using calipers in the ultrasound device or via specialised software. The first few days of an ICU admission is a busy time when clinical procedures and investigations are common. To limit time in the patient's bedspace during this time, the decision was made to acquire all the ultrasound images, save them onto a USB, and then analyse the ultrasound images at a later date using the online software program, National Institutes of Health (NIH) Image J version 1.52 (US NIH, Maryland, MD, USA). Muscle thickness was measured as the

*Images from ICU-Muscle study participants (K.Lambell)

distance (cm) from the superficial fat-muscle interface to the relevant bony landmark, or inner muscle fascia layers for the abdominis rectus muscle[100]. Rectus femoris CSA was measured by tracing around the inner muscle fascia layer. See example ultrasound images and muscle thickness and CSA measurements in Figure 2.8. The mean value from the three images at each site were used for analysis.

Reliability testing of ultrasound protocol

A range of reliability testing was performed for the ultrasound protocol. Intra-rater reliability for the entire ultrasound protocol was undertaken by Candidate, KL, repeating the landmarking and image acquisition in the final 10 study patients. Specifically, the entire protocol was performed, marks were removed and then the protocol was repeated (in the same session). The muscle thicknesses for each set of images were then compared. Intra-rater reliability for muscle thicknesses occurred by KL re-analysing images for 10 study patients at least 6 months after the initial analysis.

Inter-rater reliability for landmarking and image acquisition was assessed in 5 separate healthy volunteers (rather than ICU patients and/or study participants because of the nature of the ICU environment and to limit patient burden). The volunteers were positioned in an ICU bed with the head of the bed at 30 degrees. Firstly, Candidate KL performed the protocol, and the marks were then removed; and the second research team member (JW) followed directly after. The muscle thicknesses for each set of images were then compared. Inter-rater reliability for the quantification of muscle thicknesses occurred by having an independent trained operator (Louise Becroft, Dietitian at Alfred Health and PhD candidate) undertake a second analysis of images for a randomly selected subgroup of five participants from the study cohort.

2.2.7.3 Bioimpedance spectroscopy

Bioimpedance spectroscopy (BIS) was performed directly after the ultrasound protocol. The ImpediMed SFB7 device (ImpediMed Limited, Pinkenba, QLD, Australia) was used. The following standardised procedures were followed (as recommended by Earthman)[68]:

- Patient positioning: supine with head of the bed at 30° (usual positioning in our ICU). Arms separated >30° from the trunk and legs separated by ~45°. Where necessary towels were used to separate arms from trunk and legs from each other.
- The BIS device was placed on a nonmetal surface at least one metre away from electronic or magnetic devices.
- The dorsal surface of the hands and feet were cleaned with alcohol wipes and device-specific electrodes were placed 5cm apart: two on the hands and two on the feet.
- The leads were attached to the electrodes and the measurement recorded (at 10 second intervals for one minute).

Note: Early enteral nutrition is an accepted standard of care and is routine practice at The Alfred ICU with most patients having received enteral nutrition within the first 48 hours of ICU admission. For this reason, BIS measurements were performed regardless of fasting status. Other standard pre-test BIS procedures such as no smoking and exercise, and explicitly voiding prior to a BIS measurement were not applicable in the critically ill cohort (most participants had indwelling catheters for urine excretion).

After the first measurement, the leads were removed. At least five minutes later, with the patient in the same position, the leads were reconnected and a second measurement was taken. The data were then uploaded to the Impedimed software program (Bioimp, version

5.5.0.1, Impedimed Ltd, Pinkemba, Qld, Australia) and the modelled results, including raw data (including phase angle), and extracellular water (ECW), intracellular water (ICW), total body water (TBW), and fat-free mass (FFM, kg), were then exported into a password protected Excel spreadsheet (Microsoft Corp., Redmoind, WA, USA) for further interpretation. The SFB7 derives FFM by dividing TBW by 0.732. The mean values from each set of two measurements were used for analysis.

To avoid including in the analysis any raw FFM values that were not reflective of muscle mass status (e.g., extreme fluid overload) a measurement was accepted and used for analysis if it met the following criteria: Cole plot followed a half semi-circular pattern, standard error of estimation (SEE) for fit to the curve below 1.0, intracellular resistivity (Ri) *greater* than extracellular resistivity (Re), and whole body FFM within physiological limits (e.g. none of the water or FFM values larger than body weight)[119]. The software fitted the resistance and reactance spectral data to a semi-circular Cole model, from which key model terms were derived and applied to the software algorithm using the default analysis parameters which included data from 10 to 500 kHz, and automatic correction for time delay (i.e., high frequency capacitance). Rejection limits (up to 10%) were applied in an attempt to exclude outliers when SEE were > 1.0 and data were included if all the criteria were met after these limits were applied.

A *fluid-adjusted* FFM (kg) variable was also calculated using the Chamney equation, using the mean ECW and ICW values as follows[103]:

- FFM-Chamney = (2.725*ICW) + (0.191*Chamney Excess Fluid) - (0.191*weight). With Chamney Excess Fluid = (1.136*ECW) - (0.430*ICW) - (0.114*weight)

2.2.7.4 Subjective physical assessment of muscularity

Prior to ultrasound and BIS measurements, the physical assessment template of the subjective global assessment (SGA) tool was used to subjectively identify muscle depletion at multiple anatomical sites, Table 2.1. Critically ill patients are often bedridden and attached to medical equipment at ICU admission, which largely limited the site of muscle assessment to the temple, clavicle, shoulder, quadriceps, calf, and knee. The patient was recorded as having muscle wasting if the majority of findings were in the mild-moderate or severe muscle wasting columns.

Table 2.1 Subjective global assessment template for observing the level of muscle depletion. Adapted from Detsky et al[63]

Anatomical area	No sign	Mild-moderate	Severe
Temple	Well-defined muscle	Slight depression	Hallowing, depression
Clavicle	Not visible in Males; may be visible but not prominent in females	Some protrusion; may not be all the way along	Protruding/prominent bone
Shoulder	Rounded	No square look; acromion process may protrude slightly	Square look; bones prominent
Scapula/ribs[a]	Bones not prominent; no significant depressions	Mild depressions or bone may show slightly; not all areas	Bones prominent; significant depressions
Quadriceps	Well rounded; no depressions	Mild depression	Depression; thin
Calf	Well developed		Thin; no muscle definition
Knee	Bones not prominent		Bones prominent
Interosseous muscle[a]	Muscle protrudes; could be flat in females		Flat or depressed area

[a]Sites often not possible to assess for muscle depletion in bedridden ICU patients

2.2.7.5 Arm anthropometry

Mid-upper arm circumference (MAC, cm) was measured at the mid-point between the tip of the acromion and the olecranon process (at same site that was marked for the mid-upper arm ultrasound measurement, section 2.2.7.2), while the patient was laying supine with head of bed at 30° and using standard operating procedures[122]. The bedside nurse lifted the patient's arm, and then arm circumference was measured at the marked site using a non-stretch tape measure (being careful not to pinch or gap the tape). The measurement was recorded to the nearest 0.1cm. This method has been previously used in critically ill patients, where MAC <15th percentile was able to predict mortality and major complications (i.e. sepsis and multiorgan failure)[122].

Triceps skinfold thickness (mm) was also measured using Harpenden skinfold calipers (John Bull, British Indicators Ltd, United Kingdom), which were applied to the posterior surface of the fully relaxed and lifted arm, at the same marked point at the mid-upper arm[16]. Where possible, the right arm was used. If it was not possible to use the right arm (e.g, due to trauma), the left arm was used. Measurement was recorded to the nearest millimetre, and converted to centimetres for analysis. Mid-arm muscle circumference (MAMC, cm) was calculated using mid-upper arm circumference and triceps skinfold thickness, using the following formula:

- *Mid-arm muscle circumference (cm) = mid-upper arm circumference (cm) – (3.142 x tricep skinfold thickness [cm])*

Where possible, measurements were taken on the right side (or left if right was not available). Two measurements were taken at each point and the average used for analysis.

2.2.8 Identification of patients with low muscularity

Muscularity status (normal or low) was determined using published thresholds for each of the methods of muscle assessment, as follows:

- CT image analysis: low CT muscle CSA was classified using cut-points derived from a general ICU population where low CT muscle CSA was associated with increased mortality (CSA <170cm^2 males and CSA <110cm^2 females)[7]
- BIS: international guideline thresholds for low FFM index (FFM divided by height in metres squared) were used (FFM index <17kg/m^2 males and FFM index <15kg/m^2 females)[62]
- Arm anthropometry (MAC and MAMC): there are limited data available to guide

the most appropriate threshold to define low muscularity using arm anthropometry in critical illness. Hence, the decision was made to classify participants with low muscularity if the value was <15th percentile using the age and sex-specific data from the 2007-2010 National Health and Nutrition Examination Survey (NHANES), which was the most recent data set with tricep skin fold measurements[121]. This value was chosen based on a previous study which reported that MAC <15th percentile predicted mortality in a group of critically ill patients[122]

- Subjective physical assessment: low muscularity was recorded for participants who displayed mild-moderate or severe muscle wasting using the physical assessment section of the SGA tool[63]

2.2.9 Patient characteristics

Age, sex, primary reason for admission, time and date of hospital and ICU admission, time and date of CT scan, location before admission and co-morbidities were all collected via the patient's electronic medical record. The Acute Physiologic and Chronic Health Evaluation (APACHE) II[137] and III[138] score at ICU admission were also collected (via Alfred ICU research database) to indicate severity of illness on admission. The maximum APACHE II score is 71, with a score of 25 and >35 representing predicted mortality of 50% and 80%, respectively [137].

Using information in the electronic medical record, the Charlson Co-morbidity Index, which is a tool used to indicate overall health on admission, was calculated[139]. The index is predictive of one-year mortality based on age and a range of comorbid conditions, such

as cancer, diabetes or heart disease (total of 22 conditions). Each condition is given a score of 1, 2, 3, or 6, depending on the risk of mortality associated with each one. A score of zero indicates a younger person (<50 years) with no co-morbidities. A higher score means a stronger prediction that the outcome will result in mortality or higher healthcare resource use. ICU and hospital length of stay and hospital mortality were also recorded using data from The Alfred ICU research database.

Weight (kg) recorded was the pre-admission weight obtained from the patient or family or estimated by visual assessment by the researcher. Height (m) was either reported by the family or estimated. For descriptive purposes, BMI (kg/m^2) was calculated by dividing weight by the square of height in metres. BMI category was determined using the World Health Organisation BMI cut-off values (underweight = <18.5kg/m^2, normal weight= 18.5-24.9kg/m^2, overweight= 25-29.9kg/m^2, obese= >30kg/m^2)[140].

Prior to performing the ultrasound and BIS protocol, the research team performed a subjective assessment of signs of fluid overload (no sign, moderate or severe) by physical examination. Fluid balance in 24 hours prior to performing the bedside protocol was also recorded using data from the medical record (input minus output).

2.2.10 Outcome measures

Primary outcome (to address thesis objective 1)

- Correlation between ultrasound-derived muscle layer thickness (mid-upper arm, forearm, rectus abdominus, and quadriceps musculature, cm) and CT-derived skeletal muscle CSA (cm^2) at the L3 area.

Secondary outcome variables:

- Correlation between ultrasound-derived rectus femoris CSA (cm^2) and CT-derived skeletal muscle CSA (cm^2) at the L3 area (thesis objective 1).
- Correlation between BIS variables (FFM (kg), normally-hydrated FFM (kg), and phase angle) and CT-derived skeletal muscle CSA (cm^2) at the L3 area (thesis objective 3).
- Correlation between arm anthropometry (MAC and MAMC, cm) and CT-derived skeletal muscle CSA (cm^2) at the L3 area (thesis objective 3).
- Agreement between bedside classification of muscularity (normal or low) and CT-measured muscle status (normal or low) (thesis objectives 2 and 4).

2.2.11 Ethical considerations

The main ethical issues for this study were: the enrolment of participants who are unable to provide informed consent themselves, co-enrolment with other studies, and patient burden when taking the measurements. A full ethics application was submitted and approved by The Alfred Ethics Committee (12/12/2016). Expedited Human Research Ethics Committee approval was then obtained from La Trobe University. Approval certificates are available in Appendix 1.

2.2.12 Sample size

A sample size calculation comparing two different muscle mass indices (measured at different anatomical areas) is challenging. As highlighted by Bland and Altman, an approach to assess the agreement between two methods of a clinical measurement, is to

choose the sample size to estimate the 95% limits of agreement [141,142]. Based on this, Bland and Altman recommend that generally 100 participants is a good sample size[142].

In a study comparing DXA-measured lean tissue to ultrasound-derived muscle thickness[85], Paris et al used an online calculator developed by Dr David Schoenfeld (Professor in Department of Biostatistics and Professor of Medicine, Harvard Medical School), to determine a sample size. Specifically, a two-tailed test (α=0.05) was performed using standard deviations, SD, for knee extensor measured using MRI (muscle volume = 374cm^3 [SD 84]) and ultrasound (muscle thickness = 0.9cm [SD 47])[98]. From this, a sample size of 97 was calculated based on 80% power, with a minimal detectable difference of 120cm^3 (which was calculated using a regression equation to predict knee volume using minimal change in ultrasound-derived muscle thickness of 0.3cm).

From the above data, it was estimated that around 100 patients would be required to power the ICU-Muscle study. Recruiting this number of critically ill patients at ICU admission was deemed by the research team as not feasible within an appropriate timeframe. With the aim of completing recruitment targets within two-years (to reduce the occurrence of major changes in clinical practices or testing equipment during the recruitment) a pragmatic sample size of 50 patients was chosen and the study deemed a pilot study as it may not have been of sufficient sample size to achieve statistical significance in the primary outcome.

2.2.13 Statistical analysis

The candidate, KL, with consultation and support from supervisors and the university allocated biostatistician, performed the statistical analysis. Statistical Package for the Social Sciences (SPSS) version 25 (IBM, Armonk, New York, USA) was used for all analyses.

To compare muscularity assessed by bedside methods and CT image analysis at ICU admission (thesis objectives 1 and 3) the following statistical analyses were performed:

- Independent samples t-tests were used to assess differences in mean CT muscle CSA and bedside methods.
- Pearson correlation was used to assess the relationship between CT muscle CSA and bedside methods (ultrasound muscle thicknesses [cm], BIS-FFM [kg], Chamney (normally hydrated)-FFM [kg], Phase Angle, MAC [cm], MAMC [cm]).

For ultrasound (chapter 3) the additional statistical approaches were undertaken:

- Stepwise linear regression was undertaken to identify the ultrasound model (including baseline covariates) with the strongest correlation with CT muscle CSA. All possible combinations of the sum of ultrasound-derived muscle thicknesses at each landmark and baseline covariates that had a significant independent association with CT muscle CSA ($p<0.001$) were assessed. The best-performing ultrasound model was chosen based on the number of data points (indicating feasibility) and the strength of the relationship with CT muscle CSA assessed by Pearson's correlation.
- Bland-Altman analysis were then performed to compare CT-measured muscle CSA to ultrasound-predicted CT muscle CSA (values derived from the best-performing ultrasound protocol)[141]. This type of analysis is considered to be important to the

body composition assessment field when comparing techniques and is described in more detail below[68]. *Note*: Because CT and ultrasound-derived values are not the same parameter (e.g. like DXA FFM (kg) and BIS FFM (kg)) then the classic approach to Bland-Altman analysis wasn't possible. Therefore, CT muscle CSA was compared to ultrasound-predicted CT muscle CSA using the best-performing ultrasound protocol.

- The first step in this type of analysis is to plot the mean differences between values generated by two methods of measurement on the y-axis against the average of the values produced by the two methods on the x-axis[68]. Following this, the next step is to take the mean difference (i.e. bias) between the methods and calculate the limits of agreement around the mean bias. This is done by calculating the mean ±1.96 standard deviation (SD) for the differences between the methods and drawing a horizontal line corresponding to the mean, to the value at the mean +1.96 SD, and to the value at the mean -1.96 SD[68]. The limits of agreement should encompass 95% of all measured values (assuming a normal distribution). The width of the limits of agreement have been described as an indicator of the precision of the technique being assessed (with narrow limits of agreement, around a mean bias of zero indicating a high level of precision)[68]. However, as highlighted by Earthman, a criticism of Bland and Altman analysis is that the decision to accept the new technique (e.g. bedside body composition technique) as an acceptable alternative to the reference method, is subjective and left up to the evaluator, which can introduce variability in interpreting the clinical relevance of the individual

biases[68]. Another important step is to determine if the differences between the values are significantly correlated with the averages (see below).

- Linear regression analysis was performed for the differences against the averages with a P value <0.05 indicating proportional bias (a trend to higher or lower values, i.e. suggesting that the bedside method consistently underestimates or overestimates the measured variable compared with the reference method)[68].

Two different methods were employed to assess the specificity and sensitivity of the bedside method to accurately classify patients as having normal or low CT muscle CSA (thesis objectives 2 and 4), see below:

- For ultrasound (Chapter 3) receiver operating characteristic (ROC) curve (area under the curve, AUC) analysis was undertaken. As outlined earlier in the introduction (section 1.4), this statistical approach is often used to assess the accuracy of a diagnostic test/device/method, by plotting the true positive rate (sensitivity) against the false positive rate (1- specificity)[71]. In general, an AUC value of 0.5 suggests no discrimination (no diagnostic ability), 0.7 to 0.8 is considered acceptable, 0.8-0.9 is considered excellent, and more than 0.9 is considered outstanding (perfect diagnostic ability)[71].
- For BIS, arm anthropometry, and subjective physical assessment (Chapter 4) the sample size was smaller so Cohen's kappa statistic was used to evaluate the agreement between muscularity status determined by the bedside and CT image analysis. The Kappa result has been suggested to be interpreted as follows: values ≤ 0 as indicating no agreement and 0.01–0.20 as none to slight, 0.21–0.40 as fair, 0.41– 0.60 as moderate, 0.61–0.80 as substantial, and 0.81–1.00 as almost perfect agreement[143]

To understand whether taking the mid-upper arm muscle thickness measurement at the study patient's dominant arm influenced measurements and correlations, the mean muscle thickness measurements of those taken at the dominant and non-dominant arm were compared used paired t-tests. Correlations between mid-upper arm muscle thickness (using dominant and non-dominant measurements) and CT muscle CSA were performed using Pearson's correlation.

To assess the intra- and inter-rater reliability of the ultrasound and CT protocols for the measurement of muscle indices, both the coefficient of variation and intraclass correlation coefficient were used.

2.3 Application of computed tomography scans to investigate changes in muscle mass and quality according to week of critical illness and the association with energy and protein delivery: a retrospective study

2.3.1 Study design

This study was a retrospective, observational, single-centre cohort study that used clinical data documented as part of routine clinical care obtained from The Alfred Hospital electronic medical records. The study was designed to address thesis objectives 5-7 (see Table 1.9).

2.3.2 Study aims and hypotheses

The aims of this study were to:

1. Explore changes in CT-measured skeletal muscle mass and quality (density) according to week of critical illness.
2. Investigate associations with energy and protein delivery and other clinical variables thought to influence changes in muscularity.
3. Explore the precision of CT image analysis to detect changes in muscle mass and quality.

The primary study hypothesis was that significant loss of skeletal muscle mass and quality during ICU admission (>5%) would be observed and greater energy and protein delivery (and adequacy compared to dietitian estimates) would be associated with attenuation of skeletal muscle mass and quality.

2.3.3 Study setting

This study used data from patients admitted to The Alfred ICU (see characteristics of The Alfred ICU in section 2.2.4).

2.3.4 Study population

Participants who were admitted to The Alfred ICU from February 2009 until July 2019 and who had two or more CT scans performed at the abdominal area between seven and 21 days apart were screened for inclusion. The rationale for choosing the date range was that patient data and scans were not readily available before 2009.

2.3.5 Patient eligibility

Inclusion criteria

- Patients ≥18 years old and admitted to The Alfred ICU from February 2009 until July 2019 who had 2 or more CT scans (including L3) performed between seven and 21 days apart, either ≤24hrs before or during ICU admission. The seven-day minimum interval between scans was chosen as sufficient to detect changes in muscularity if they were to occur; and
- Patients who were predominantly fed via the enteral and/or parenteral route (planned >70% estimated requirements) in the time between the CT scans.

Patients were excluded if:

- The CT scan was not appropriate for analysis for skeletal muscle CSA assessment (i.e., if borders between specific tissues are indistinguishable, a proportion of muscle CSA is cut off); and/or

- The plan was to feed more than 30% of energy and protein requirements via the oral route at any stage during the study period. This is because oral intake is not routinely recorded in a quantifiable manner at The Alfred ICU, which would influence accuracy of quantification of energy and protein delivery and thus our findings of the association between nutrition delivery and changes in muscularity.

2.3.6 Patient recruitment

The Alfred Health Information System Manager was asked to extract details of patients who had two or more CT scans (≥ 7 days apart) performed in ICU (between February 2009 and July 2019). The candidate, KL, then screened all potential patients for eligibility using the electronic medical record and criteria outlined above. The date and week of ICU admission that the first CT scan was performed was recorded (week 1: day 0-7, week 2: day 8-14, weeks 3-4: day 15-28, weeks 5-7: day 29-49).

2.3.7 Study procedures

2.3.7.1 CT image analysis

CT-measured skeletal muscle CSA was measured at L3, using the same methodology as described in section 2.2.7.1.

In addition to measurement of muscle CSA, the sliceOmatic software also automatically computed *skeletal muscle density* by calculating the mean radiological muscle attenuation of all muscle visible at the L3 level, measured in Hounsfield Units, HU. As discussed in the

introduction, this measurement can be influenced by the administration and phase of contrast during CT scanning[59,75]. Therefore, scans were excluded from skeletal muscle density analyses if the comparator scans did not have similar contrast administration (as determined by an experienced radiologist, A/prof Gerard Goh).

Short-term precision

The short-term precision of CT image analysis to assess changes in skeletal muscle CSA and density was evaluated by identifying and collecting data for 10 adult ICU patients who had two CT scans at the L3 area for clinical purposes performed ≤24 hours apart (a period where significant changes would not be expected). A sample of ten patients was chosen to identify significant differences in measured muscle area and density if there were going to be any. The patients were identified by The Alfred Health Information Services manager, going back 10 years, and then including the first 10 patients where the CT scans were analysable. *Note*: these group of patients were an entirely separate cohort of patients to the main cohort.

2.3.8 Patient characteristics and clinical variables

The same patient variables were recorded as outlined in 2.2.9, including: age, sex, Charlson Comorbidity Index[139], Acute Physiologic and Chronic Health Evaluation (APACHE) II[137] and III[138] score, admission diagnosis (trauma, medical or surgical), ICU and hospital LOS, and in-hospital mortality.

2.3.8.1 Anthropometry

At The Alfred Hospital, ICU-trained dietitians assess every patient who commences enteral and/or parenteral nutrition. This assessment, which is documented in the medical records, was used to collect the participant's weight, height, and BMI (kg/m^2). During the data extraction period ICU patients were not routinely weighed (as often not feasible due to lack of weigh beds), so the patient's weight (and height) was commonly reported by the patient or family members or estimated by the dietitian. For patients with a BMI $\geq 30kg/m^2$ an adjusted body weight (actual body weight - ideal body weight [weight at BMI 25] + 25% excess weight) was used to calculate energy and protein requirements and delivery per kilogram body weight (as recommended by ICU nutrition clinical guidelines)[18].

2.3.8.2 Daily clinical variables thought to impact changes in muscularity

As highlighted in chapter 1 (section 1.2.2), the causes for muscle wasting are multifactorial and may be influenced by severity of illness, organ failure, immobility, and energy and protein delivery[26]. Variables thought to influence changes in muscle mass that were available in the patient's medical record were collected for each day in between the comparator CT scans. As outlined in Table 2.2, these included daily requirements for organ support, medications reflecting severity of illness, level of mobility, and nutrition delivery (which is described in more detail below).

Table 2.2 Patient characteristics and clinical and nutrition variables collected

Baseline	**Each day between comparator CT scans**
Age	Mechanical ventilation (*yes/no*)
Gender	CRRT (*yes/no*)
Weight *(kg)*	ECMO (*yes/no*)
Height *(m)*	Sedation (*yes/no*)
Body Mass Index (BMI)	Insulin (*yes/no*)
APACHE II and APACHE III	Vasopressors and/or inotropes (*yes/no*)
Charlson co-morbidity index	ICU mobility scale (*0-10*)
Reason for admission	Estimated energy requirement (*kJ*)
Pre-ICU admission location	Estimated protein requirement (*g*)
	Delivered energy [EN/PN, dextrose & propofol] (*kJ*)
	Delivered protein [EN/PN] (*g*)

Nutrition requirements and delivery

Energy (kilojoules) and protein (grams) requirements were recorded for each day between the CT scans using estimates determined by the patient's ICU dietitian(s) managing the patient's nutrition care (and not involved in the study). Specifically, at The Alfred Hospital, energy requirements are typically determined using the Schofield equation and adding a stress/injury factor as necessary[144], and protein requirements are set according to clinical guideline recommendations (e.g. 1.3 g protein per kg/day)[18] . An adjustment for obesity as described in 2.3.8.1 above is also common practice.

At The Alfred Hospital, nursing staff document hourly volumes of enteral and/or parenteral nutrition delivered to patients in the patient's medical record. Delivered energy and protein were calculated from all possible sources (enteral nutrition [EN], parenteral nutrition [PN], intravenous dextrose, propofol, and intravenous amino acid [synthamin]). Volumes of EN discarded due to intolerance (high gastric residual volume) were subtracted from the delivered volume.

Daily adequacy (%) was calculated for both energy and protein calculated as: (daily amount delivered) / (daily estimated requirements) x 100. As the number of study days were not consistent for all patients, energy and protein adequacy was calculated as the total adequacy divided by the number of study days. To enable the provision of energy and protein in this study to be compared to other studies and to clinical guideline recommendations, mean energy and protein delivered per kilogram actual or adjusted body weight (for patients with BMI ≥30kg/m^2, see 2.3.8.1) averaged per study day were also calculated for each patient.

2.3.9 Outcome measures

Primary outcomes (to address thesis objective 7):

- Change in CT-measured skeletal muscle CSA (cm^2) between comparator scans (% change per study day).

Secondary outcomes:

- Change in CT-measured skeletal muscle density (HU) between comparator scans (% change per study day) (thesis objective 7).
- Correlation between energy delivery and adequacy and changes in CT-measured skeletal muscle CSA (cm^2) and density (HU) (thesis objective 5).
- Correlation between protein delivery and adequacy and changes in CT-measured skeletal muscle CSA (cm^2) and density (HU) (thesis objective 5).
- Correlation between study days receiving medications/organ support and changes in CT-measured skeletal muscle CSA (cm^2) and density (HU) (thesis objective 5).

To explore the precision of CT image analysis to detect changes in muscle mass and quality in critical illness (thesis objective 6) the outcome was:

- Difference between CT-measured skeletal muscle CSA (cm2) and density (HU) between the CT scans performed <24hrs apart (expressed as percentage difference).

2.3.10 Ethical considerations

A low-risk ethics application was submitted and approved by The Alfred Human Ethics Committee. Expedited Human Research Ethics Committee approval was then obtained from La Trobe University. All the information obtained and analysed in this study was collected as part of routine clinical care. This study did not affect patient care and all patient information was coded and kept in a password-protected database (only accessible by the research team). As such, there was no known or likely reason for thinking that participants would not have consented if they had been asked. For these reasons, a waiver of consent was requested and approved. The ethics approval certificates are available in Appendix 1.

2.3.11 Sample size

At the time of conceptualisation of the retrospective study there were only two studies that investigated the relationship between energy and protein delivery and CT-measured skeletal muscle changes in critically illness[48,50]. Braunschweig et al. studied 33 patients with respiratory failure who were a sample of convenience, where data were collected for consideration in a larger randomized controlled feeding trial[48]. The authors of this study reported an overall decline in abdominal muscle CSA over the study period (mean 10

days). However, this was not statistically significant in both men (*p*=0.07) and women (*p*=0.09). In univariate regression, greater energy delivery was the only significant predictor of the percentage change in muscle mass (β=0.024, *p*=0.03)[48]. The second study, Casaer et al., studied 15 neurosurgical patients randomised to early (n=10) or late parenteral nutrition (n=5)[50]. The participants, who at the time they had a CT brain scan performed for clinical purposes, were then re-positioned to scan the abdomen area. The CT scans were performed on median ICU day 2 and ICU day 9[50]. This study reported no significant change in abdominal muscle volume loss over the study period in both the control and intervention group[50].

Due to the lack of adequately powered studies investigating the association of energy and protein delivery on skeletal muscle changes in critically ill patients, we included all eligible patients going back 10 years where CT scans and medical records were readily available, the CT scanning methodology was relatively consistent, and medical records were readily available.

2.3.12 Statistical analysis

The Candidate (KL), with consultation and support from supervisors and co-investigators, performed the statistical analysis. Statistical Package for the Social Sciences (SPSS) version 25 (IBM, Armonk, NY, USA) and Stata SE version 15.0 (StataCorp, College Station, TX, USA) were used.

To explore changes in CT-measured skeletal muscle CSA (cm^2) and density (HU) according to week of critical illness (thesis objective 7) patients were grouped according to the week

that the first CT was performed and percentage changes in both indices were calculated and expressed as change per day. A paired samples t-test was used to assess differences in skeletal muscle CSA and density between the two comparator scans. A repeated measures analysis was also performed in patients who had >2 CT scans by fitting a linear mixed model to calculate the mean change in CT-measured muscle CSA and specifying the number of weeks since admission as the fixed effect and patient as random effect.

To investigate the relationship between changes in muscularity and energy and protein delivery and other nutrition variables (thesis objective 5), the percentage change in CT-measured skeletal muscle CSA and density per study day, calculated from the first to the last scan, were used. Associations between changes in skeletal muscle CSA and density and the clinical and nutrition variables were assessed by Pearson or Spearman correlations.

To explore the precision of CT image analysis to detect changes in muscle mass and quality in critical illness (thesis objective 6), a paired samples t-test and coefficient of variation were used to assess differences in CT-measured skeletal muscle CSA and density of the two scans (performed <24 hours apart).

2.4 Conclusion

This chapter has outlined the methods used in the two original research studies in this thesis. Chapter 5 outlines the methodology and results for the systematic literature review. The following chapters (3, 4, and 6) discuss the results of the two studies.

Chapter 3

Can ultrasound be used to assess muscularity at ICU admission?

A PROSPECTIVE OBSERVATIONAL STUDY

3.1 Declaration of authorship

Student's declaration:

The nature and extent of contributions to Chapter 3 of this thesis are as follows:

Name	Nature of contribution	Contribution
Kate Lambell	Study concept and design, ethics application, participant consent, data collection and analysis, data interpretation, and manuscript preparation and revision for publication	70%
Audrey Tierney	Study design, data analysis and interpretation, and revision of manuscript	5%
Jessica Wang	Data collection and revision of manuscript	3%
Vinodh Nanjayya	Study design, data analysis and interpretation, and revision of manuscript	3%
Adrienne Forsyth	Study design, data analysis and interpretation, and revision of manuscript	5%
Gerard Goh	Data collection and analysis and revision of manuscript	1%
Don Vicendese	Data analysis and interpretation and revision of manuscript	1%
Emma Ridley	Data interpretation and revision of manuscript	1%
Selina Parry	Study design, data interpretation, and revision of manuscript	1%
Marina Mourtzakis	Study design, data interpretation, and revision of manuscript	1%
Susannah King	Study design, data analysis and interpretation, and revision of manuscript	9%

Supervisor's declaration:

I hereby certify that the declaration above is a correct reflection of the extent and nature of contributions made toward Chapter 3 of this thesis by the student and all listed co-authors.

Name of supervisor	**Signature**
Susannah King	

3.2 Introduction

Low muscularity at intensive care unit (ICU) admission has been associated with increased length of stay and mortality and therefore may be an important predictor of outcome[7-9]. The quantification of muscle mass is pivotal to the assessment of nutritional status whereby muscle atrophy is strongly related to malnutrition[62]. Furthermore, body composition analysis is important to consider for the determination of nutrition requirements (with fat-free mass being the largest driver of metabolic rate) and for monitoring the effectiveness of nutrition interventions aimed at attenuating muscle wasting[18]. Despite the importance of assessing muscularity in acute illness, there is currently no routinely available bedside tool that is considered sufficiently reliable and accurate to objectively estimate whole-body muscularity in the ICU setting. As such, the development of bedside methods to assess muscularity has been identified a key research priority for monitoring nutritional status and predicting functional recovery in critical care[65].

This chapter describes a pilot observational study (ICU-Muscle) aimed at evaluating whether ultrasound can provide a quantifiable assessment of muscularity and identify individuals with low muscularity at ICU admission. This was a stand-alone study designed and carried out specifically for the candidate's doctoral research. The chapter relates to thesis objectives 1 and 2:

- To compare muscularity assessed by a multi-site bedside ultrasound protocol and CT image analysis at ICU admission.
- To investigate the ability of the ultrasound protocol to identify patients with low CT-measured muscularity.

The methods for this study are outlined in detail in Chapter 2. The results for this chapter are presented in the form of a manuscript accepted and published in the *Journal of Parenteral and Enteral Nutrition (Impact Factor 4.016), the citation is as follows:*

> Lambell KJ, Tierney AC, Wang JC, Nanjayya V, Forsyth A, Goh GS, Vicendese D, Ridley EJ, Parry SM, Mourtzakis M, King SJ. Comparison of ultrasound-derived muscle thickness with computed tomography muscle cross-sectional area on admission to the intensive care unit: a pilot cross-sectional study. *JPEN*. 2021 Jan;45(1):136-45. DOI: 10.1002/jpen.1822

As at 17th July 2021, the article has been cited 7 times (via Google Scholar). The findings were also accepted for an oral presentation at the Australasian Society for Parenteral Nutrition (AuSPEN) annual scientific meeting in Adelaide, November 2019. An abstract was also accepted for a poster presentation at the American Society for Parenteral and Enteral Nutrition (ASPEN) Nutrition Science and Practice Conference, Tampa, Florida 2020 (Appendix 3). Due to the COVID-19 pandemic the conference was cancelled hence a poster was not presented. However, the abstract was published in the supplementary file (S29, page 102) in the following publication:

> ASPEN NUTRITION SCIENCE & PRACTICE CONFERENCE: March 28-31, 2020, Tampa, Florida: Vars Candidates, Trainee Awards, Best of ASPEN (Topic Awards), International Awards, Abstracts of Distinction, Posters of Distinction and Other Abstracts. *JPEN J Parenter Enteral Nutr*. 2020;44(2):382.

The supplementary data (a table associated with the publication) are presented after the manuscript. The table displays the correlations between ultrasound muscle thickness

measurements (at each landmark and combination) and CT muscle CSA. These analyses were undertaken to support making a decision on the best-performing optimal protocol, which was chosen based on the number of data points (indicating feasibility) and the strength of the relationship with CT muscle CSA. This “optimal” protocol was then compared to CT muscle CSA using Bland and Altman analyses.

At the end of the chapter the results of additional analyses (not included in the publication) are presented:

- Correlations between ultrasound-measured rectus femoris CSA and CT muscle CSA (section 3.4.1); and
- Data to understand the influence of using the dominant arm for mid-upper arm muscle thickness measurements (section 3.4.2).

3.3 Manuscript

"A comparison of ultrasound-derived muscle thickness with computed tomography muscle cross-sectional area on admission to the intensive care unit: A pilot cross-sectional study"

Original Communication

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Comparison of Ultrasound-Derived Muscle Thickness With Computed Tomography Muscle Cross-Sectional Area on Admission to the Intensive Care Unit: A Pilot Cross-Sectional Study

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Abstract

Introduction: The development of bedside methods to assess muscularity is an essential critical care nutrition research priority. We aimed to compare ultrasound-derived muscle thickness at 5 landmarks with computed tomography (CT) muscle area at intensive care unit (ICU) admission. Secondary aims were to (1) combine muscle thicknesses and baseline covariates to evaluate correlation with CT muscle area and (2) assess the ability of the best-performing ultrasound model to identify patients with low CT muscle area. *Methods:* Adult patients who underwent CT scanning at the third lumbar area <72 hours after ICU admission were prospectively recruited. Muscle thickness was measured at mid-upper arm, forearm, abdomen, and thighs. Low CT muscle area was determined using published cutoffs. Pearson correlation compared ultrasound-derived muscle thickness and CT muscle area. Linear regression was used to develop ultrasound prediction models. Bland-Altman analyses compared ultrasound-predicted and CT-measured muscle area. *Results:* Fifty ICU patients were enrolled, aged 52 ± 20 years. Ultrasound-derived muscle thickness at each landmark correlated with CT muscle area ($P < .001$). The sum of muscle thickness at mid-upper arm and bilateral thighs, including age, sex, and the Charlson Comorbidity Index, improved the correlation with CT muscle area ($r = 0.85$; $P < .001$). Mean difference between ultrasound-predicted and CT-measured muscle area was −2 cm^2 (95% limits of agreement, −40 cm^2 to +36 cm^2). The best-performing ultrasound model demonstrated good ability to identify 14 patients with low CT muscle area (area under curve = 0.79). *Conclusion:* Ultrasound shows potential for assessing muscularity at ICU admission (Clinicaltrials.gov NCT03019913). (*JPEN J Parenter Enteral Nutr.* 2021;45:136–145)

Keywords

body composition; computed tomography; critical illness; intensive care unit; skeletal muscle mass; ultrasound

Clinical Relevancy Statement

Currently, there is no routinely available bedside tool that is considered reliable and accurate for objectively assessing whole-body muscularity in the intensive care unit (ICU) setting. The primary aim of this prospective study was to evaluate the relationship between muscularity assessed by

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bedside ultrasound with a reference method (single-slice computed tomography [CT] image analysis) on admission to the ICU. The sum of ultrasound-derived muscle thickness at the mid-upper arm and thighs was strongly correlated to CT muscle area. These results demonstrate the potential for ultrasound to assess muscularity on admission to the ICU.

Background

Low muscularity at intensive care unit (ICU) admission has been associated with increased length of stay (LOS) and mortality, and therefore, may be an important predictor of outcome.[1-3] The quantification of muscle mass is pivotal in the assessment of nutrition status whereby muscle atrophy is strongly related to malnutrition.[4] Further, body composition analysis is important to consider for the determination of nutrition requirements (with fat-free mass being the largest driver of metabolic rate) and for monitoring the effectiveness of nutrition interventions aimed at attenuating muscle wasting.[5]

Despite the importance of assessing muscularity in acute illness, there is currently no method that is considered accurate, reliable, and feasible in the ICU setting.[6] Reference methods for body composition analysis, such as dual-energy x-ray absorptiometry (DXA) and computed tomography (CT) image analysis, are costly, often inaccessible, involve radiation (CT), and are impractical for use in critically ill patients, often requiring patients to be transported out of the ICU for measurement.[7] Ultrasound is an emerging tool for the assessment of muscularity in the ICU setting largely because it is safe, noninvasive, portable, and readily available in most ICUs.[8] There are only limited data evaluating the utility of ultrasound as a measure of muscularity in critically ill patients, finding a moderate correlation between ultrasound-derived quadriceps muscle thickness and CT muscle cross-sectional area (CSA) using maximal compression ultrasound technique.[9] In healthy volunteers, ultrasound protocols incorporating measurements of the upper and lower limbs and using minimal compression technique have reported a strong agreement with fat-free mass assessed by DXA.[10,11]

Therefore, we aimed to compare ultrasound-derived muscle thickness at 5 different anatomical landmarks, with muscularity assessed by a reference method that is accessible in a subgroup of critically ill patients on ICU admission (CT muscle CSA at the third lumbar [L3] area). Our secondary aims were to (1) evaluate if combining muscle thickness at different landmarks and readily available patient information could strengthen the correlation with CT muscle area and (2) to assess the ability of the best-performing ultrasound model to accurately classify patients with low CT muscle area.

Methods

Patients

This was a prospective observational study conducted in a single center between January 23, 2017, and March 25, 2019, after approval from the research and ethics committees at The Alfred Hospital and La Trobe University. The study was registered a priori on clinicaltrials.gov (NCT03019913). Patients were screened on predetermined weekdays when investigators were available and met inclusion criteria if they were aged $\geq$18 years and had a CT scan including the L3 area performed for clinical purposes $\leq$24 hours before or $\leq$72 hours after ICU admission. Exclusion criteria included the following: unanalyzable CT scan, imminent death, anticipated ICU stay of $<$24 hours, pregnancy, and impracticality and/or incapability to perform the ultrasound protocol (including imaging of at least 2 or more muscle groups, including at least 1 thigh) or to obtain consent. Patients with a body mass index (BMI) of $>$40 kg/m^2 were also excluded, as they were outside the range for previously assessed utility of a similar ultrasound protocol in the ICU setting.[12]

Written and informed consent was obtained from the eligible patients and/or their legal medical decision maker. For all patients, the following demographic and clinical data were collected: age, sex, weight, height, Charlson Comorbidity Index,[13] Acute Physiology and Chronic Health Evaluation (APACHE) II[14] and III[15] scores, admission diagnosis (trauma, medical, or surgical), ICU and hospital LOS, and in-hospital mortality. BMI (kg/m^2) was calculated using estimated or reported weight and height on ICU admission, and BMI category was determined using the World Health Organization BMI cutoff values (underweight, $<$18.5 kg/m^2; normal weight, 18.5–24.9 kg/m^2; overweight, 25–29.9 kg/m^2; and obese, $\geq$30 kg/m^2).[16]

CT Image Analysis

During the screening process, investigators visualized skeletal muscle area at L3, and where necessary, a consultant radiologist (G.G.) confirmed the quality of the scan was adequate for analysis. Patients were excluded if the muscle borders were indistinguishable; there was interference of artifact; and/or if the whole muscle group(s) was not visible because of positioning during CT scanning.

CT scans were uploaded onto the licensed software, SliceOmatic version 5.0 (TomoVision, Montreal, QC, Canada) for analysis by investigator K.J.L., who identified the L3 area and the CT slice for analysis. Skeletal muscle boundaries were recognized based on Hounsfield units (–29 to +150).[17] Abdominal skeletal muscle CSA (cm^2), herein termed *CT muscle area*, was automatically computed by the software by summing the skeletal muscle-tissue pixels and multiplying by the surface area of each pixel.

Intrarater reliability for CT image analysis was performed by the primary investigator (K.J.L.) relandmarking and reanalyzing scans from 10 study patients at least 6 months after initial analysis. Interrater reliability was performed by having a second trained investigator (L.M.) landmark and analyze scans from 10 study patients.

Ultrasound

Trained investigators (K.J.L. or J.C.W.) performed the one-off evaluation of muscularity by ultrasound as soon as possible after patient enrollment. The sites chosen to compare with CT muscle area included muscle thickness of the right mid-upper arm and forearm (left side if right not available), abdomen, and bilateral thighs (details below). The sites were chosen because they are readily accessible while a patient is supine. Further, the measurement protocols for determining muscle thickness at the upper and lower limbs have been reported as reliable in the ICU setting and associated with whole-body muscularity in healthy volunteers.[10,11,18-20] It was hypothesized that including ultrasound assessment of a muscle group at the L3 region may strengthen agreement between the 2 methods, and therefore, rectus abdominis muscle thickness was included.

A portable B-mode ultrasound device (Philips Sparq, Philips Ultrasound, Bothell, WA, USA) with a multifrequency linear array transducer (4–12 MHz) was used. Patients were supine with the head of the bed at approximately 30 degrees (usual positioning in our ICU). Water-soluble transmission gel was applied to the transducer. Using minimal compression, the transducer was held perpendicular to the skin at the mark on the skin, and depth was adjusted to visualize the relevant bone (or the inner muscle fascia layers for the abdomen). Three still images were taken at each landmark and saved and uploaded to the National Institutes of Health (NIH) Image J software for analysis (version 1.52, US NIH, Maryland, MD, USA). The previously published measurement protocols for each anatomical site are described below.

Mid-upper arm. A mark was made on the skin at the midway point between the tip of the acromion and the olecranon process. The thickness of the bicep flexor compartment was imaged with the elbow extended and forearm supinated and resting on the bed. Muscle thickness was measured from the subcutaneous adipose tissue–muscle interface to the muscle-bone interface of the humerus.[12,19]

Forearm. A point was marked at 30% proximal between the ulnar styloid process and the head of the radius. With the hand supinated and forearm relaxed on the bed, the image was taken. Ulna muscle thickness was measured as the distance between the subcutaneous adipose tissue–muscle interface and muscle-bone interface of the ulna.[21]

Abdominal. A mark was made 3 cm to the right of the umbilicus. The probe was rotated, and the image saved as the rectus abdominis muscle was positioned horizontally on the screen. Muscle thickness was measured from the distance between the upper and lower inner muscle fascia layers (in the center of the image).[21]

Bilateral thighs. With knees extended and relaxed, a point was marked at the anterior superior iliac spine and the upper pole of the patella. A point was then marked at the midpoint and two-thirds point between these landmarks. Muscle thickness was measured from the subcutaneous adipose tissue–muscle interface to the muscle-bone interface of the femur at both points on both thighs.[18]

For each site, the average result of the 3 still images was used for analysis. For each thigh, the value used for analysis was an average of muscle thickness at the midpoint and two-thirds point. The bilateral thigh thickness value was taken as the average across both thighs (ie, [right midpoint + right two-thirds + left midpoint + left two-thirds]/4). For upper arm, forearm, and thigh, muscle thickness (cm) was multiplied by limb length (distance between each bony landmark (eg, acromion and the olecranon process for upper arm) (cm), and this value used for analysis, as previously described.[10]

A range of reliability testing was performed for ultrasound. Intrarater reliability for the protocol was undertaken by investigator K.J.L. repeating the landmarking and image acquisition in the final 10 patients. The muscle thicknesses for each set of images were then compared. Intrarater reliability for muscle thicknesses occurred by K.J.L. reanalyzing images for 10 participants at least 6 months after the initial analysis. Interrater reliability for the ultrasound protocol (landmarking and image acquisition) was assessed in 5 separate healthy volunteers (rather than ICU patients because of the nature of the study environment and to limit participant burden). The volunteers were positioned in an ICU bed with the head of the bed at 30 degrees. The first investigator (K.J.L.) performed the protocol, and the marks were then removed; and the second investigator (J.C.W.) followed directly after. The muscle thicknesses for each set of images were then compared. Interrater reliability for the quantification of muscle thicknesses occurred by having an independent operator (L.B.) undertake a second analysis of images for a randomly selected subgroup of 5 from the ICU patient cohort.

Statistical Analyses

For this pilot study, a pragmatic sample size of 50 patients was chosen based on predicted eligibility, with the aim of completing recruitment targets within a 2-year time frame to reduce the occurrence of major changes in clinical practices or testing equipment during the recruitment period. Shapiro-Wilk tests were used to assess normality. Data are

reported as n (%), mean and standard deviation (± SD), or median and interquartile range [IQR]. Missing data were not imputed.

Differences in mean CT muscle area and ultrasound-derived muscle thickness by sex and age (<65 years vs ≥65 years) were assessed using independent samples *t*-tests.[9] Pearson correlation was used to assess the relationship between CT muscle area and ultrasound measures. Baseline covariates thought to influence the level of muscularity (age, sex, BMI, and Charlson Comorbidity Index) were individually assessed for their relationship with CT muscle area by univariate linear regressions. Stepwise linear regression was undertaken to identify the ultrasound model with the strongest correlation with CT muscle CSA, including all possible combinations of the sum of ultrasound-derived muscle thickness at each landmark and baseline covariates that had a significant independent association with CT muscle CSA ($P < .001$). The best-performing ultrasound model was chosen based on the number of data points (indicating feasibility), the strength of the relationship with CT muscle area, and limits of agreement determined by Bland-Altman analyses (95% limits of agreement for differences between ultrasound-predicted and CT-measured muscle area).[22] To assess the limits of agreement, linear regression analysis was performed for the differences against the averages, with a *P*-value < 0.05 indicating proportional bias (a trend to higher or lower values).[7]

Muscularity status (normal or low) was determined using published CT muscle area cutoff values (<170 cm^2 for men and <110 cm^2 for women) derived from a general ICU population in which low CT muscle area was associated with increased mortality.[1] Receiver operating characteristic curve analysis was undertaken to assess the specificity and sensitivity of the optimal ultrasound model to accurately classify patients as having normal or low CT muscle area (using ultrasound-predicted CT muscle area generated from the best-performing ultrasound model).

Intraclass correlation coefficient (ICC) and coefficient of variation (CV) were used to assess intrarater and interrater reliability. IBM SPSS version 25 (Armonk, NY) was used for all analysis, and significance was set at a *P*-value of <0.05.

Results

A total of 1580 patients were screened, and of the 373 patients who had a CT scan including the L3 area, 323 patients were excluded, and 50 patients were included (Figure 1). Participants were predominantly male (38 [76%]) and admitted post trauma (42 [84%]), with a mean age and median APACHE II score of 52 ± 20 years and 12 [9–16], respectively. Other characteristics are detailed in Table 1.

The mean time from ICU admission to performing the ultrasound protocol was 33 ± 12 hours and

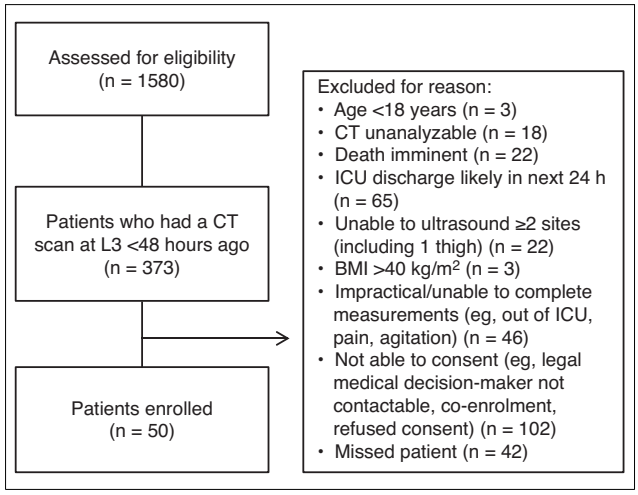

Figure 1. Consort diagram for the inclusion process. BMI, body mass index; CT, computed tomography; ICU, intensive care unit; L3, third lumbar.

Table 1. Patients' Clinical and Demographic Characteristics.[a]

Characteristics	All patients (n = 50)
Age, y	52 ± 20 (21–88)
Age category	
<65 years	33 (66)
≥65 years	17 (34)
Sex	
Male	38 (76)
Female	12 (34)
APACHE II	12 [9–16] (2–36)
APACHE III	45 [35–65] (17–139)
Height, m	1.72 ± 0.09 (1.50–1.98)
Weight, kg	82 ± 15 (50–120)
BMI, kg/m^2	28 ± 5 (18–38)
Underweight	1 (2)
Normal weight	15 (30)
Overweight	18 (36)
Obese	16 (32)
Charlson Comorbidity Index	2 ± 2 (0–6)
Admission reason	
Trauma	42 (84)
Multitrauma (excluding head)	29 (69)
Multitrauma (including head)	4 (10)
Traumatic brain injury	9 (21)
Medical	7 (14)
Surgical	1 (2)
Patients receiving MV	31 (62)
ICU LOS, d	5 [2–11] (1–36)
Hospital LOS, d	16 [11–24] (3–61)
Hospital mortality	4 (8)

APACHE, Acute Physiology and Chronic Health Evaluation; BMI, body mass index; ICU, intensive care unit; LOS, length of stay; MV, mechanical ventilation.

[a]Values are reported as mean ± SD (range); median [interquartile range] (range) or n (%).

26 ± 13 hours from CT scan to the ultrasound protocol. The mean CT muscle area was 173 ± 38 cm^2, with males having significantly higher muscle area than females (187 ± 29 cm^2 vs 127 ± 26 cm^2; $P < .001$), as did those who were younger (<65 vs ≥65 years old) (189 ± 30 cm^2 vs 141 ± 32 cm^2; $P < .001$) (Table 2).

Of the 50 patients included, ultrasound images were available for the following number of patients at each site: 48 for mid-upper arm, 39 for forearm, 39 for abdominal, 49 for 1 thigh, and 37 for bilateral thighs. The mean muscle thicknesses for the individual sites and according to sex and age category are outlined in Table 2. Reasons for missing ultrasound data, which largely relate to traumatic injuries, are presented in Table 3. Arm measurements on the right side were not accessible from a small number of patients because of pain or traumatic injury, and the left side was used (4 for mid-upper arm and 3 for forearm).

Reliability of Measurement Protocols

The method of CT image analysis showed good reliability with intrarater testing revealing CV = 0.7% and ICC = 0.998 and interrater testing revealing CV = 0.8% and ICC = 0.995. For the ultrasound protocol, because of the study environment (first few days of ICU admission) and requirement for clinical procedures, it was only possible to repeat the protocol in 6 patients (not 10 as planned). The ultrasound protocol also showed good reliability for 1) relandmarking and image acquisition (intrarater CV = 2.8% and ICC = 0.966; interrater CV = 3.8% and ICC = 0.997), and 2) muscle thickness measurements (intrarater CV = 2.2% and ICC = 0.998, and interrater CV = 3.6% and ICC = 0.992).

Comparison Between Ultrasound-Derived Muscle Thickness and CT Muscle Area

There was a significant positive relationship between ultrasound-derived muscle thickness at each anatomical landmark and CT muscle area (mid-upper arm, $r = 0.79$; forearm, $r = 0.68$; 1 thigh, $r = 0.70$; both thighs, $r = 0.75$; and abdomen, $r = 0.68$) ($P < .001$). The sum of muscle thickness at the mid-upper arm and bilateral thighs (or 1 thigh if not able to image both thighs) was the ultrasound protocol that had the most complete data (n = 47), a strong positive relationship to CT muscle area ($r = 0.82$; $P < .001$) (Figure 2A), and underwent further evaluation incorporating baseline covariates as outlined below. Supplementary Table S1 provides a summary of the correlations between ultrasound muscle thicknesses at each landmark (and combination) and CT muscle area.

Table 2. Characteristics of CT Muscle Area And Ultrasound-Derived Muscle Thickness at Each Landmark and by Sex and Age Group.

Variable	All (n, mean ± SD)		Male		Female	*P*-value	Young (<65 years)		Older (≥65 years)		*P*-value
CT muscle CSA, cm^2	50	172.9 ± 38.2	38	187.3 ± 29.2	12	127.4 ± 26.0	.001	33	189.1 ± 30.5	17	141.5 ± 32.2	.001
Mid-upper arm, cm^{2a}	48	109.2 ± 27.8	36	119.2 ± 23.0	12	79.3 ± 17.9	.001	31	119.4 ± 25.3	17	90.4 ± 22.3	.001
Forearm, cm^{2a}	39	112.4 ± 23.2	30	119.1 ± 21.6	9	90.1 ± 11.3	.001	25	120.2 ± 20.1	14	98.6 ± 21.0	.004
One thigh, cm^{2a}	49	155.1 ± 49.2	37	169.8 ± 38.0	12	109.7 ± 53.2	.003	32	176.4 ± 37.5	17	114.9 ± 43.7	.001
Bilateral thighs, $cm^{2a,b}$	49	154.8 ± 47.9	37	169.0 ± 37.0	12	111.2 ± 52.6	.003	32	177.4 ± 35.2	17	112.3 ± 39.2	.001
Abdomen, cm	39	1.0 ± 0.3	33	1.1 ± 0.3	6	0.7 ± 0.4	.003	26	1.2 ± 0.3	13	0.7 ± 0.3	.001

CSA, cross-sectional area; CT, computed tomography.
[a] Muscle thickness (cm) multiplied by limb length (cm).
[b] Average muscle thickness of bilateral thighs (or muscle thickness for 1 thigh if images not available for both).

Table 3. Reasons for Missing Ultrasound Data.

Reason for missing data	Thigh (n = 13)	Mid-upper arm (n = 2)	Forearm (n = 11)	Abdominal (n = 11)
Traumatic injury	6	2	2	0
Lines/dressings	0	0	6	1
Wounds	1	0	0	8
Unanalysable image	2	0	3	1
Other	4	0	0	1

Incorporation of Baseline Covariates

Baseline covariates with a significant independent association with CT muscle area were age ($r = 0.53$; $P < .001$), sex ($r = 0.66$; $P < .001$), and Charlson Comorbidity Index ($r = 0.54$; $P < .001$). BMI did not have a significant association with CT muscle area ($r = 0.23$; $P = .104$) and was therefore not included in further modeling. Incorporating age, sex, and Charlson Comorbidity Index to the ultrasound protocol further strengthened the relationship with CT muscle area ($r = 0.85$; $P < .001$), and this combination was labeled the best-performing ultrasound model (Figure 2B). The mean difference between CT-measured and ultrasound-predicted CT muscle area generated from the best-performing ultrasound model was -2 cm^2 (95% limits of agreement, -40 to $+36$ cm^2), with no proportional bias ($P = .102$); see Figure 3.

Identification of Participants With Low Muscularity

Fourteen participants (10 men and 4 women) were identified as having low CT muscle area.[1] Using ultrasound-predicted CT muscle area derived from the best-performing ultrasound model (n = 47), 85% of patients were correctly classified as having normal or low CT muscle area, with 79% sensitivity and 94% specificity. The positive predictive and negative predictive values were 82% and 86%, respectively. The best-performing ultrasound model had good ability to identify patients with low CT muscle area (area under the curve [AUC] = 0.79 [95% CI, 0.65–0.92]) (Figure 4).

Discussion

To our knowledge, this is the first study to compare muscularity assessed by ultrasound at multiple anatomical sites with a reference method for muscle assessment in critically ill patients. We compared ultrasound-derived muscle thickness measured at the mid-upper arm and thighs at ICU admission with CT muscle area at the L3 region and found a strong correlation. The addition of age, sex, and Charlson Comorbidity Index strengthened the relationship and accounted for 70% of the variance in muscle assessed by CT image analysis. The mean bias between measured and ultrasound-predicted CT muscle area was -2 cm^2 with limits of agreement from $+36$ to -40 cm^2. There is currently no consensus on what is considered acceptable performance in terms of prediction of muscularity at the individual level, but our data provide a reference point for comparison with subsequent studies.

Although most of the ICU literature using ultrasound has focused on describing changes in muscle thickness and/or muscle CSA at ICU admission[23,24] and using it as a tool to monitor the responsiveness of nutrition interventions,[25,26] there is a paucity of literature evaluating the accuracy of ultrasound measurements of muscularity compared with reference methods in the critical care setting. This is primarily due to the challenges of performing traditional body composition methods in critical care. When other reference methods are unavailable or inaccessible, CT image analysis at the L3 area is considered to be a useful method; however, because of cost and radiation exposure, scan acquisition is generally restricted to clinical diagnostic indications, and therefore, the study populations in ICU using this method are likely to represent only a subset of the broader mixed ICU population. This further highlights the need for the validation of bedside tools that can measure body composition in a wide range of critically ill patients.

Most frequently in the ICU literature, muscle ultrasonography has focused on the quadriceps group, which is proposed to have more considerable implications on physical and clinical outcomes compared with other muscle groups.[8] However, the findings from the current study demonstrate that ultrasound measurement of the thigh alone may not provide the most optimal representation of whole-body muscularity. These results are supported by a recent study by Paris et al in 96 healthy volunteers in which ultrasound-derived muscle thickness of bilateral quadriceps alone had a strong relationship to appendicular lean tissue mass assessed by DXA ($R^2 = 0.72$) but was further improved by adding anterior mid-upper arm muscle thickness and age and sex covariates ($R^2 = 0.92$).[10] Further, critically ill patients lose muscle at differing rates from different areas of the body, and therefore, when considering a tool to measure the effectiveness of interventions aimed to attenuate

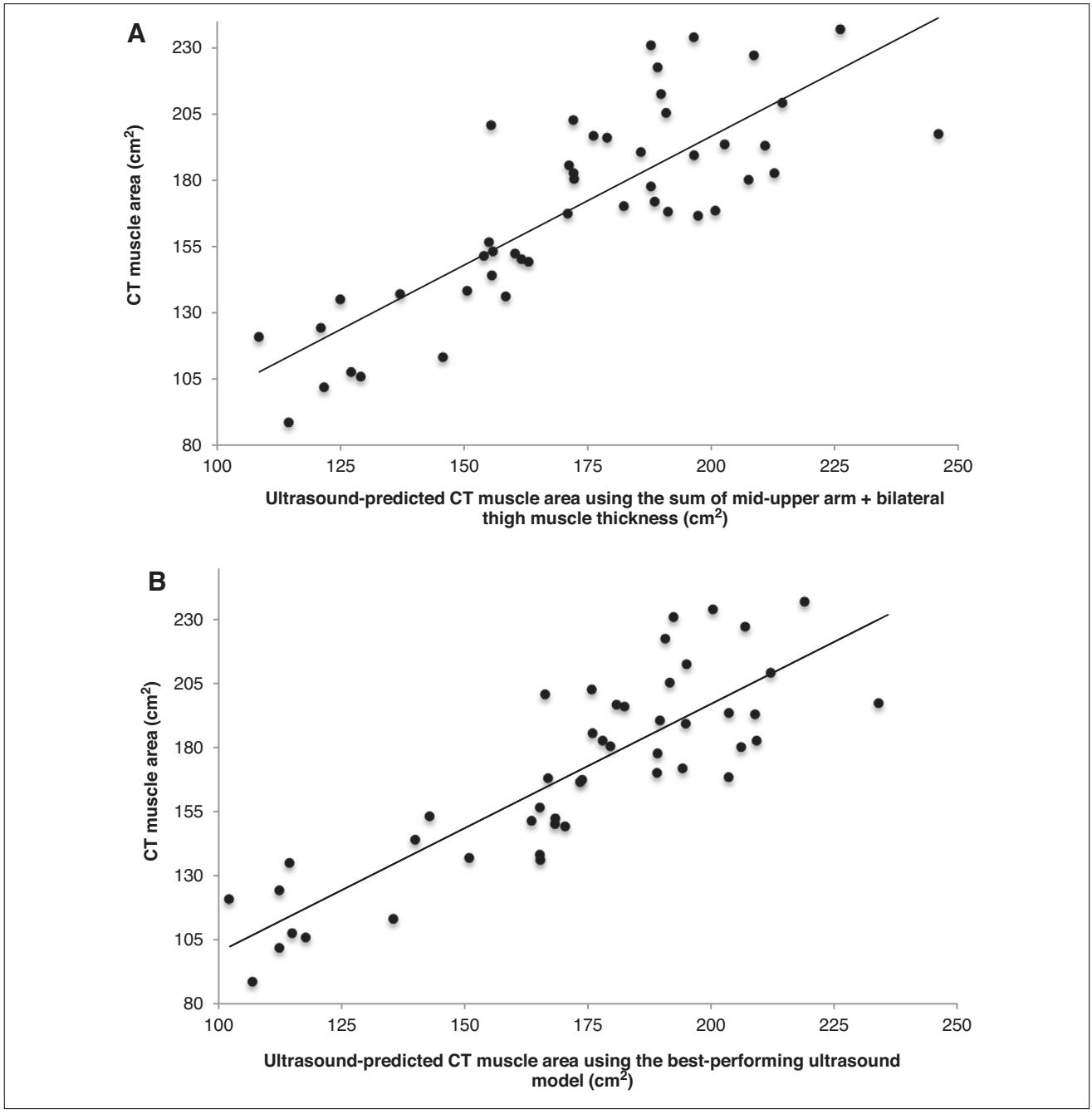

Figure 2. Correlation between CT muscle area and ultrasound-predicted CT muscle area using sum of mid-upper arm + bilateral thigh muscle thickness (A: $r = 0.82$, adjusted $R^2 = 0.66$, $P < 0.001$), and with ultrasound-predicted CT muscle area using the best-performing ultrasound protocol (including sex, age, and Charlson Comorbidity Index) (B: $r = 0.85$, adjusted $R^2 = 0.70$, $P < 0.001$). The solid line is the best-fit regression line. CT, computed tomography.

whole-body muscle wasting (such as nutrition delivery), it may be important to consider the assessment of muscle groups at both the upper and lower limbs.[27,28]

Low muscularity and malnutrition have been associated with a range of adverse clinical outcomes in the acute setting, and patients identified as malnourished may benefit from more intensive nutrition therapy.[29,30] The diagnosis of malnutrition using criteria set out in the recent Global Leadership Initiative on Malnutrition (GLIM) recommendations[4] and in the widely used subjective global assessment (SGA) tool are challenging in the ICU setting,[31] specifically because these assessments rely on obtaining an

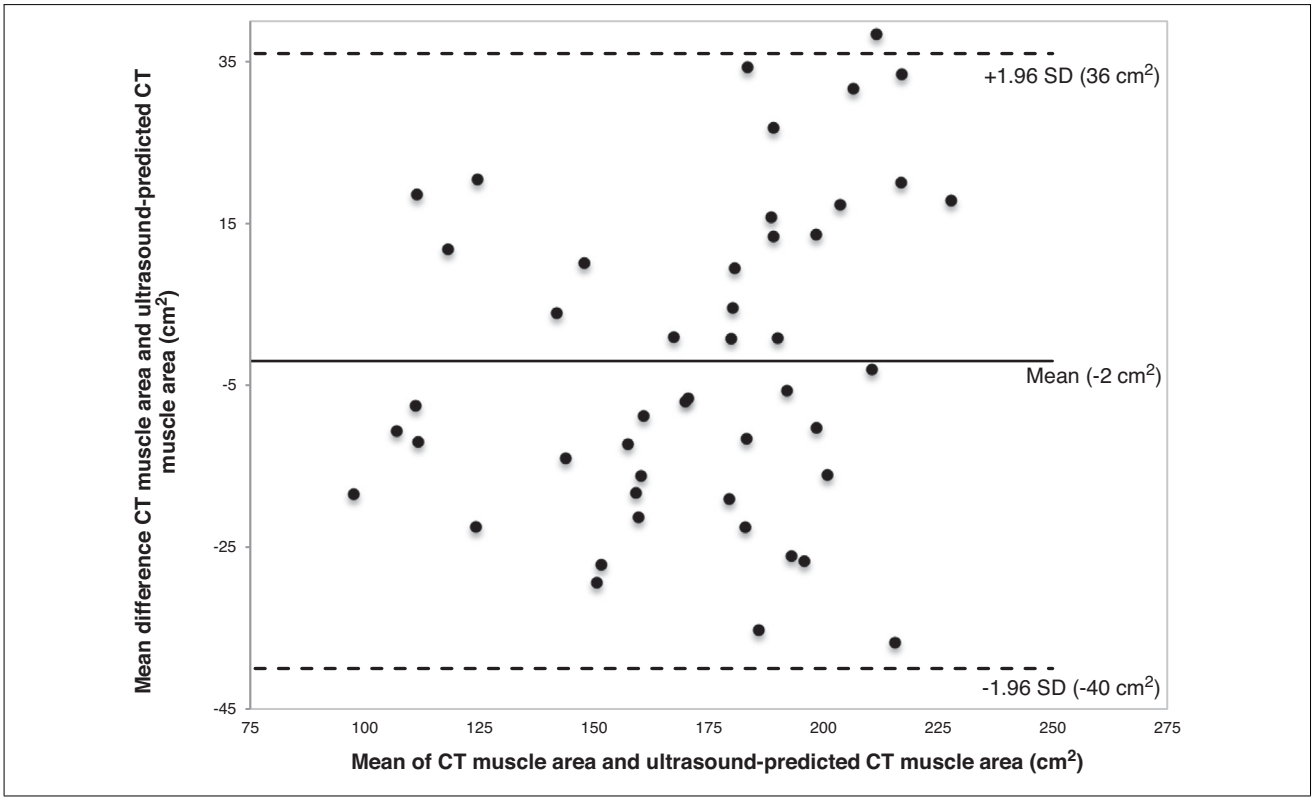

Figure 3. Bland-Altman analysis between CT muscle area and ultrasound-predicted CT muscle area (using best-performing ultrasound model)(cm^2). The solid line represents the mean bias, and the dashed lines represent the limits of agreement (+ 1.96 SD) between the paired measurements. CT, computed tomography.

accurate weight and weight history. These are frequently affected by fluid overload and an inability to obtain a history from the patient early in the ICU admission. Additionally, the remaining part of the SGA tool involves dietary history and subjective physical assessment of muscle and fat wasting, the latter of which is also recommended by GLIM when reference body composition methods are not available and may also be affected by edema and obesity. These challenges were demonstrated in 56 ICU patients who also had a CT scan at the L3 area.[32] All were classified as normally nourished by a dietitian using SGA, but despite this classification, 56% had low muscularity on CT image analysis.[32] Therefore, it is highly relevant for the assessment of nutrition status to consider the ability of an objective bedside method to classify a patient as having low or normal muscularity accurately. This is supported by the GLIM recommendations in which the identification of depleted muscle stores is included as a criterion for the diagnosis of malnutrition.[4] The ultrasound model described in this study demonstrated a good ability to accurately classify the 14 patients with low CT muscle area (AUC = 0.79). Although the sample size was small, this finding highlights that ultrasound may be a useful tool to identify patients with muscle wasting who may be malnourished on ICU admission and to quantitatively monitor muscularity during the ICU and hospital stay.

There are no internationally recognized cutoff values for classifying patients with low muscularity using ultrasound-derived muscle thickness. Recently, in the study aforementioned, Paris et al developed cut points for ultrasound muscle thickness at the thigh and anterior mid-upper arm to classify individuals into 3 groups (low, moderate, and high) for risk of low lean-tissue mass.[10] Given the present study used a similar protocol, these cut points may warrant further investigation to determine whether they have relevance to functional and clinical outcomes in ICU patients.

This study has strengths and limitations, which need to be considered. A strength is the high acquisition rate for ultrasound of the upper arm and thighs even in a cohort largely composed of trauma patients, demonstrating its feasibility as a bedside body composition method on ICU admission. This study was performed in a single center, which fosters consistency in the application of ultrasound technique to test its capabilities to reliably assess muscularity. Further, the ultrasound protocol was efficient to perform (<30 minutes) and trainable for nonmedical professionals, which highlights the potential for widespread use of the method. Limitations include the modest sample size.

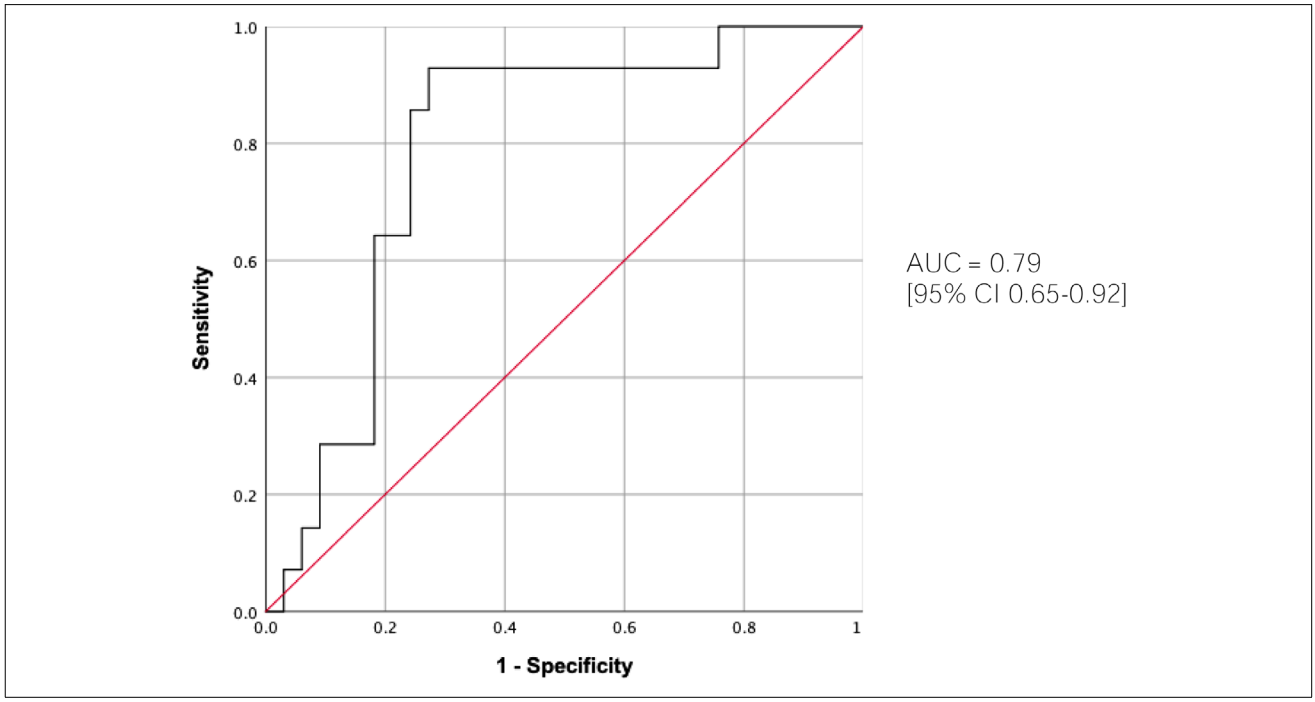

Figure 4. Receiver operator characteristic curve. AUC, area under the curve.

Caution should be exercised in generalizing the results to the broader ICU population, given the high representation of trauma patients in our sample (due to the inclusion requirement for patients having a CT scan). It remains unknown whether CT muscle area determined by a single slice at the L3 area is representative of whole-body muscle in ICU patients.

Conclusion

Ultrasound has the potential to assess muscularity and to identify patients with low muscle mass on ICU admission. Although the results from this study need extension in other settings and tracking over time, we have demonstrated a strong relationship between muscularity assessed with a widely available and applicable ultrasound method and a reference method. Future research priorities include investigating how muscle status, assessed by ultrasound on ICU admission, relates to important functional and clinical outcomes.

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Statement of Authorship

K. J. Lambell contributed to the conception and design of the research; S. J. King, A. C. Tierney, A. Forsyth, E. J. Ridley, S. M. Parry, M. Mourtzakis, and V. Nanjayya contributed to the design of the research; K. J. Lambell, J. C. Wang, and G. S. Goh contributed to the acquisition and analysis of the data; K. J. Lambell, S. J. King, A. C. Tierney, and D. Vicendese contributed to the interpretation of the data; and K. J. Lambell drafted the manuscript. All authors critically revised the manuscript, agree to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final manuscript.

Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Supplementary data (table associated with the manuscript)

Table 3.1 Correlation between ultrasound muscle thickness measurements (at each landmark and combination) and CT muscle area

Individual ultrasound site	**n**	**Muscle thickness multiplied by limb length (cm^2) (mean±SD)**	**Correlation with CT muscle area (Adjusted R^2 value)**
Mid-upper arm (A)	48	109.21 ± 27.84	0.612[a]
Forearm (B)	39	112.42 ± 23.15	0.441[a]
One thigh (C)	49	155.08 ± 49.16	0.481[a]
Both thighs (D)	37	153.86 ± 50.94	0.605[a]
Both thighs (or one if other n/a) (E)	49	154.82 ± 47.86	0.548[a]
Abdominal (F)	39	1.03 ± 0.34[b]	0.445[a]
Combinations - sum of ultrasound sites			
A + B	39	224.27 ± 45.73	0.657[a]
A + C	47	261.59 ± 70.39	0.612[a]
A + D	35	257.96 ± 71.63	0.678[a]
A + E	47	261.57 ± 69.70	0.658[a]
A + B + C	38	379.12 ± 85.31	0.664[a]
A + B + D	27	374.98 ± 85.85	0.692[a]
A + B + E	38	379.12 ± 83.55	0.702[a]
B + C	38	269.13 ± 64.37	0.559[a]
B + D	27	265.73 ± 67.16	0.607[a]
B + E	38	268.13 ± 62.44	0.606[a]
A + F	37		0.719[a]
B + F	32		0.611[a]
C + F	38		0.641[a]
D + F	31		0.578[a]
E + F	38		0.618[a]
A + B + F	32		0.729[a]
A + C + F	29		0.644[a]
A + D + F	29		0.690[a]
A + E + F	38		0.678[a]
A + B + C + F	31		0.690[a]
A + B + D + F	24		0.701[a]
A + B + E + F	31		0.719[a]
B + C + F	31		0.611[a]
B + D + F	24		0.626[a]
B + E + F	31		0.667[a]

[a]*P* <0.001, [b]Abdominal muscle thickness is reported in cm (not possible to multiply by limb length). A, mid-upper arm; B, forearm; C, one thigh; D, both thighs; E, both thighs (or one thigh if other n/a); F, abdominal. *Note*: When abdominal muscle thickness was assessed in combination with other sites, it was included as an independent variable during multivariable linear regression (not included in the sum of the other sites)

3.4 Additional analyses

3.4.1 Relationship between rectus femoris CSA and CT muscle CSA

Where possible, rectus femoris CSA was measured at the two-thirds point on the right and/or left thigh. Of the 50 patients recruited, a rectus femoris CSA measurement was only possible for 27 (54%) patients. This was due to the inability to view the whole CSA of the rectus femoris muscle on one image (Figure 3.1). The reasons for this were attributable to the study population, with a large proportion of younger males many whom had high muscularity (large rectus femoris muscle) and also to the linear ultrasound probe and device used.

(A) (B)

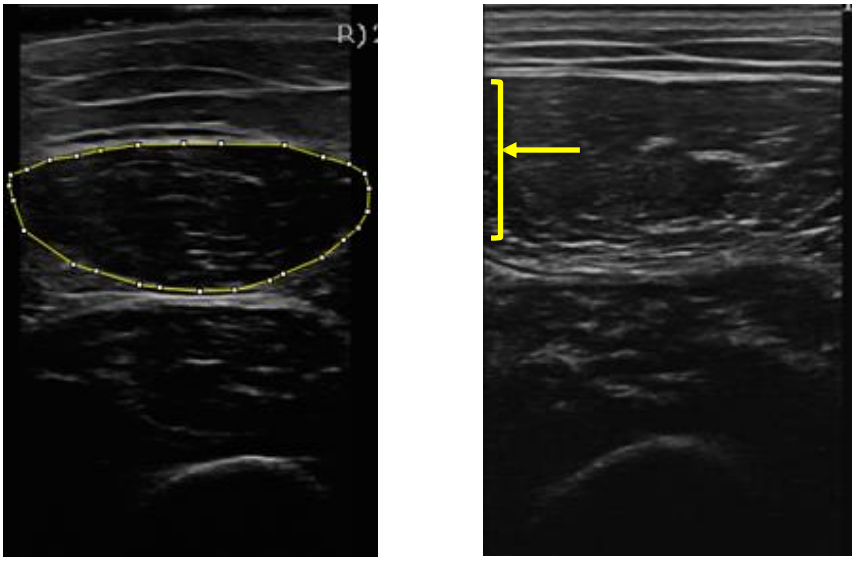

Figure 3.1. Ultrasound image at the two-thirds point on the right thigh
(A) Image of an individual where the whole rectus femoris CSA is visible (B) Image an individual with high muscularity and visualisation of whole rectus femoris CSA is not possible (vertical yellow line and arrow indicate where the muscle body is 'cut-off')

For the 27 patients in whom analysis was possible, there was a modest positive correlation between the rectus femoris CSA and CT muscle CSA (R^2= 0.257, adjusted R^2=0.227, p<0.001). Due to the low number of patients with a rectus femoris CSA measurement (also indicating low feasibility using a routinely available ultrasound device), the decision was made to exclude this site from inclusion in statistical modelling and any further analyses.

3.4.2 Dominant arm

As outlined in the methods, based on previous protocols the mid-upper arm muscle thickness ultrasound measurement was taken at the right arm (not both arms). At the time of study design, it was uncertain if taking the measurement at the study patient's dominant arm would influence the measurements and correlations with CT muscle CSA. Hence, where possible, dominant arm was recorded and additional analyses were performed to assess whether this influenced the correlations.

There were 45 patients with dominant arm recorded and a mid-upper arm muscle ultrasound measurement (cm^2, [muscle thickness, cm x limb length, cm]). Of these, there were 37 patients where dominant arm was used (82%), and 8 patients where non-dominant arm was used (22%), with mean±SD muscle thickness measurement 108.9±28.6cm^2 and 113.1±23.3cm^2, respectively indicating no significant difference between mean muscle thicknesses (p=0.698). Similar correlations between muscle thickness and CT muscle CSA were observed (dominant arm used, r=0.755; and non-dominant, r=0.878). These findings indicate that correlations were unlikely to be influenced by whether the dominant arm was imaged or not.

3.5 Conclusion

The findings presented in this chapter were that ultrasound-derived muscle thickness at the mid-upper arm and bilateral thighs were highly correlated to CT muscle CSA, and had a good ability to identify patients with low muscularity at ICU admission. This pilot data demonstrated the potential for an easily applied bedside ultrasound protocol to provide a quantifiable assessment of muscularity and identify patients with lower-than-normal muscle mass at ICU admission. Although the results from this study need extension in other settings and tracking over time, the findings demonstrate a strong relationship between muscularity assessed with a widely available and applicable ultrasound method and a reference method.

The following chapter reports secondary outcome data from the same study to investigate the relationship between other bedside techniques to assess muscularity and CT-measured muscularity.

Chapter 4

Can bioimpedance spectroscopy, arm anthropometry, and subjective physical assessment be used to assess muscularity at ICU admission?

A PROSPECTIVE OBSERVATIONAL STUDY

4.1 Declaration of authorship

Student's declaration:

The nature and extent of contributions to Chapter 4 of this thesis are as follows:

Name	Nature of contribution	Contribution
Kate Lambell	Study concept and design, ethics application, participant consent, data collection and analysis, data interpretation, and manuscript preparation and revision for publication	80%
Carrie Earthman	Study design, data analysis and interpretation, and revision of manuscript	5%
Audrey Tierney	Study design, data analysis and interpretation, and revision of manuscript	4%
Adrienne Forsyth	Study design, data analysis and interpretation, and revision of manuscript	4%
Gerard Goh	Data collection and analysis and revision of manuscript	2%
Susannah King	Study design, data analysis and interpretation, and revision of manuscript	5%

Supervisor's declaration:

I hereby certify that the declaration above is a correct reflection of the extent and nature of contributions made toward Chapter 4 of this thesis by the student and all listed co-authors.

Name of supervisor	**Signature**
Susannah King	

4.2 Introduction

This chapter describes secondary outcome data from the prospective observational pilot study (ICU-Muscle study) aimed at evaluating the ability for other easily applied bedside methods (*bioimpedance spectroscopy (BIS), arm anthropometry, and subjective physical assessment*) to provide estimates of muscularity at ICU admission, with a focus on the potential of each method to identify individuals with low muscularity at ICU admission. This chapter relates to thesis objectives 3 and 4:

- To compare muscularity assessed by bioimpedance technology, arm anthropometry, and subjective physical assessment and CT analysis at ICU admission.
- To investigate the ability of bioimpedance technology, arm anthropometry, and subjective physical assessment to identify patients with low CT-measured muscularity.

The methods employed in this study are outlined in Chapter 2 (section 2.2). The results for this chapter are presented in the form of a manuscript accepted and published in the *Journal of Human Nutrition and Dietetics (Impact factor 3.089).* As at 17th July 2021 the article has been cited 4 times. The citation is as follows:

> Lambell KJ, Earthman CP, Tierney AC, Goh GS, Forsyth A, King SJ. How does muscularity assessed by bedside methods compare to computed tomography muscle area at intensive care unit admission? A pilot prospective cross-sectional study. *J Hum Nutr Diet*. 2021 Apr;34(2):345-55. DOI: 10.111/jhn.12804

4.3 Manuscript

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ANTHOPOMETRY

How does muscularity assessed by bedside methods compare to computed tomography muscle area at intensive care unit admission? A pilot prospective cross-sectional study

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Abstract

Background: Low muscularity and malnutrition at intensive care unit (ICU) admission have been associated with negative clinical outcomes. There are limited data available evaluating the validity of bedside techniques to measure muscle mass in critically ill adults. We aimed to compare bedside methods for muscle mass assessment [bioimpedance spectroscopy (BIS), arm anthropometry and subjective physical assessment] against reference technology [computed tomography (CT)] at ICU admission.
Methods: Adults who had CT scanning at the third lumbar area <72 h after ICU admission were prospectively recruited. Bedside methods were performed within 48 h of the CT scan. Pearson's correlation compared CT muscle area with BIS-derived fat-free mass (FFM) (kg) and FFM-Chamney (kg) (adjusted for overhydration), mid-upper arm circumference (cm) and mid-arm muscle circumference (cm). Depleted muscle stores were determined using published thresholds for each method. Cohen's kappa (κ) was used to evaluate the agreement between bedside and CT assessment of muscularity status (normal or low).
Results: Fifty participants were enrolled. There were strong correlations between CT muscle area and FFM values and mid-arm muscle circumference ($P < 0.001$). Using FFM-Chamney, all six (100%) participants with low CT muscle area were detected ($\kappa = 0.723$). FFM-BIS, arm anthropometry and subjective physical assessment methods detected 28%–38% of participants with low CT muscle area.
Conclusions: BIS-derived FFM using an adjustment algorithm for overhydration was correlated with CT muscle area and had good agreement with muscularity status assessed by CT image analysis. Arm anthropometry and subjective physical assessment techniques were not able to reliably detect participants with low CT muscle area.

Introduction

Malnutrition at intensive care unit (ICU) admission has been associated with increased ICU length of stay, ICU readmission and mortality.[1] International clinical guidelines for nutrition in the critically ill recommend early nutrition therapy for malnourished patients who are admitted to the ICU.[2,3] Reduced muscle mass is highly related with malnutrition and the Global Leadership on Malnutrition (GLIM) consensus group has included reduced muscle mass in the recently published criteria for diagnosing malnutrition.[4] Furthermore, low muscularity on admission to the ICU has independently been associated with mortality and increased length of stay and may be an important predictor of outcome.[5,6] Despite the developing evidence base and guideline recommendations highlighting the importance of identifying patients with lower than normal muscularity,[4,7] few studies have evaluated how bedside body composition methods perform compared to reference technology in the ICU setting with respect to identifying this phenotype.[8]

The paucity of studies evaluating the accuracy of bedside methods to assess muscularity is primarily a result of logistical and practical challenges. Transporting critically ill patients out of the ICU for body composition assessment using a reference technology (e.g. dual-energy X-ray absorptiometry) is neither feasible, nor a clinical priority. However, in recent years, computed tomography (CT) image analysis at the third lumbar (L3) area, using scans performed for clinical diagnostic purposes has evolved as a body composition method, with skeletal muscle area being highly related to whole-body muscle.[9-11] The method is reliable and precise and is considered as a reference body composition technique for defining sarcopenia in cancer and other populations.[9] As a result, it has become possible to evaluate how bedside assessment of muscularity compares to CT-measured muscularity in critically ill patients who have had a CT scan performed for clinical purposes. CT image analysis also allows for the measurement of muscle density (a marker for muscle quality) at the L3 area, which enables the evaluation of how bedside measures relate to not only muscle mass, but also muscle quality.

The bedside tools for assessment of muscularity recommended by GLIM in general hospital populations, and supported by the European Society of Parenteral and Enteral Nutrition (ESPEN) ICU clinical guidelines, include: bioimpedance technology; subjective physical assessment of muscle stores (using a published tool); and arm anthropometry (mid-upper arm circumference, mid-arm muscle circumference).[3,4] Bioimpedance is based on the measurement of the opposition (impedance) to an electrical current by body tissues. Available technologies include single- and multi-frequency bioelectrical impedance analysis (BIA) and bioelectrical impedance spectroscopy (BIS). Variables relevant for muscularity assessment include: 50-kHz phase angle and estimates of fat-free mass (FFM) (kg).[12] Phase angle, which is generated from the arctangent of the ratio of reactance to resistance at 50 kHz, may be related to cellular health and nutrition status and has been independently associated with ICU mortality and length of stay on ICU admission.[13,14] The estimation of FFM requires the use of population-specific predictive equations, which are based on various assumptions (e.g. normal hydration of lean tissue and fluid distribution), and these are often violated in critical illness as a result of large fluid shifts and oedema.[15,16] A conceptual model (Chamney model) has been developed from cadaver data and applied to BIS data in dialysis populations, and involves an adjustment for excess fluid/overhydration based on normal hydration of lean and adipose tissue.[17] The model has been applied to fluid management in dialysis patients but has not yet been investigated in the ICU setting.[18] Subjective physical assessment and arm anthropometry techniques for muscle mass assessment may also be influenced by fluid status, and further evaluation against a reference method is required to understand the utility of the methods to assess muscularity and accurately detect muscle depletion.

The aims of this pilot study were to: (i) determine the association between bedside measures of muscle mass [BIS-derived FFM (unadjusted and fluid adjusted) and arm anthropometry] and a reference method (CT muscle area) at ICU admission; (ii) evaluate how BIS-derived phase angle relates to CT muscle area and density; and (iii) assess the agreement between muscularity status (low or normal) assessed by BIS, arm anthropometry and subjective physical evaluation of muscle stores and CT image analysis.

Materials and methods

Study design and setting

This prospective cross-sectional observational study was conducted in a single centre ICU between January 2017 and March 2019 after approval from the Research and Ethics Committees at The Alfred hospital and La Trobe University. The data presented were collected as part of a larger study.[19] The study was registered *a priori* on clinicaltrials.gov (NCT03019913). Written and informed consent were obtained from eligible patients and/or their legal medical decision-maker. Reporting of the study follows the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.[20]

Participant selection

Patients were eligible if they were aged ≥18 years and had a CT scan including the L3 area performed for clinical reasons ≤24 h *before* or ≤72 h *after* ICU admission. Exclusion criteria were: the CT scan was unanalysable for muscle assessment at the L3 area, death was imminent, anticipated ICU stay was <24 h, pregnancy, body mass index (BMI) >40 kg/m^2, it was impractical and/or not possible to complete bedside measurements or it was not possible to obtain informed consent. To limit the time between the CT scan and bedside methods, patients were also excluded if the CT scan was performed ≥48 h prior to enrolment. Baseline demographic and clinical data, including age, sex, Charlson Comorbidity Index, Acute Physiologic and Chronic Health Evaluation (APACHE) II and III score, admission diagnosis (trauma, medical or surgical), ICU and hospital length of stay, were collected for all participants [21-23]. Weight (kg) recorded was the pre-admission (dry) weight obtained from the patient or family or estimated by an experienced dietitian (KJL) by visual assessment (taking into account any apparent fluid overload). Height (m) was either reported by the family or estimated. For descriptive purposes, BMI (kgm^{-2}) was calculated. BMI category was determined using the World Health Organization (BMI) cut-off values (underweight <18.5 kg m^{-2}, normal weight 18.5–24.9 kgm^{-2}, overweight 25–29.9 kg m^{-2}, obese >30 kg m^{-2}).[24] Fluid balance in the 24-h period before performing the bedside protocol was recorded (where 24 h of data was documented in the medical record).

Computed tomography image analysis

During the screening process for eligibility, investigators visualised skeletal muscle area at L3 and, where necessary, an experienced radiologist (GSG) confirmed the quality of the scan was adequate for analysis. Patients were excluded if the muscle borders were indistinguishable, if there was interference of artifact or if whole muscle group(s) were not visible as a result of positioning during CT scanning. CT scans were uploaded onto the licensed software, SLICEOMATIC, version 5.0 (TomoVision, Montreal, QC, Canada) for analysis. A trained investigator (KJL) identified the slice for analysis at L3, and skeletal muscle boundaries were recognised based on Hounsfield units (–29 to +150 for muscle).[25] Abdominal muscle cross-sectional area (cm^2) was automatically computed by the software by summing the skeletal muscle tissue pixels and multiplying by the surface area of each pixel. Skeletal muscle density (Hounsfield units) was also automatically computed by the software by calculating the mean radiological muscle attenuation of all muscle visible at the L3 level.

Bioimpedance spectroscopy

BIS was performed within 48 h of the CT scan using the ImpediMed SFB7 BIS device (ImpediMed Limited, Pinkenba, QLD, Australia). The BIS device scans 256 frequencies between 3 and 1000 kHz and using Cole modelling and equations incorporating Hanai mixture theory, the software determines total body water (TBW), extracellular water (ECW) and intracellular water (ICW).[12] The BIS measurement was performed when the patient was supine with the head of the bed at approximately 30-degrees (usual positioning in our ICU) and with the limbs separated.[12] Participants had been bed-bound for >12 h prior to measurement. The dorsal surface of the hands and feet were cleaned with alcohol and device-specific electrodes were placed 5 cm apart: two on the hands and two on the feet. The leads were attached to the electrodes and the measurement recorded (at 10-s intervals for 1 min). The leads were then removed. At least 5 min later, with the patient in the same position, the leads were reconnected and a second measurement was taken. The data were then uploaded into the Impedimed software program (BIOIMP, version 5.5.0.1) and modelled results, including raw data, and ECW, ICW, TBW and FFM (kg), were exported into EXCEL (Microsoft Corp., Redmoind, WA, USA) for further interpretation. The mean values from the two measurements were used for analysis.

FFM (kg) was estimated from TBW measures by the Impedimed SFB7 software and was recorded as *FFM-BIS*. A modified FFM (kg) variable was also calculated using the Chamney model (equation detailed below for the Chamney 'normally hydrated lean tissue' variable), which accounts for excess fluid and is relabeled here for ease of comparisons as *FFM-Chamney* [17]:

FFM-Chamney = (2.725 × ICW) + (0.191 × Chamney Excess Fluid) – (0.191 × weight). With Chamney Excess Fluid = (1.136 × ECW) – (0.430 × ICW) – (0.114 × weight).

Phase angle at 50 kHz was also recorded.

To avoid including FFM values that were not reflective of muscle mass status (e.g. extreme fluid overload) a measurement was accepted and used for analysis if it met the following criteria: Cole plot followed a half semi-circular pattern, standard error of estimation (SEE) for fit to the curve below 1.0, intracellular resistivity (Ri) *greater* than extracellular resistivity (Re) and whole body FFM within physiological limits (e.g. none of the water or FFM values larger than body weight).[26] The software fitted the resistance and reactance spectral data to a semi-

circular Cole model, from which key model terms were derived and applied to the software algorithm using the default analysis parameters, which included data from 10 to 500 kHz, and automatic correction for time delay (i.e. high frequency capacitance). Rejection limits (up to 10%) were applied in an attempt to exclude outliers when SEE were >1.0, and data were included if all the criteria were met after these limits were applied.

Arm anthropometry

Mid-upper arm circumference (cm) was measured at the mid-point between the tip of the acromion and the olecranon process.[27] Triceps skinfold thickness (mm) was measured using Harpenden skinfold calipers (John Bull, British Indicators Ltd, Weybridge, UK), which were applied to the posterior surface of the fully relaxed and lifted arm, at the same marked point.[27] Measurement was recorded to the nearest millimetre and converted to centimetres for analysis. Mid-arm muscle circumference was calculated using mid-upper arm circumference and triceps skinfold thickness, using the formula:

Mid-arm muscle circumference (cm) = mid-upper arm circumference (cm) – [3.142 × tricep skinfold thickness (cm)]

Where possible, measurements were taken on the right side (or left if right was not available). Two measurements were taken at each point and the average used for analysis.

Physical assessment of muscle wasting

A trained investigator (KJL) undertook a subjective assessment of muscle wasting (none, mild–moderate or severe) using the physical assessment section of the widely used subjective global assessment (SGA) tool.[28]

Assessment of muscularity status

Muscularity status (normal or low) was determined using published thresholds for each of the methods. Low CT muscle area (reference method) was classified using cut-points derived from a general ICU population where low CT muscle area was associated with increased mortality (<170 cm^2 males and <110 cm^2 females).[6] For BIS, international guideline thresholds for low FFM index (FFM divided by height in metres squared) were used (<17 kg m^{-2} males and <15 kg m^{-2} females) [4,29]. Low muscularity using arm anthropometry measures was determined as <15th percentile using the age and sex-specific data from the 2007–2010 National Health and Nutrition Examination Survey[31], which was the most recent data set with tricep skin fold measurements. This value was chosen, based on a previous study which reported that mid-upper arm circumference <15th percentile predicted mortality in a group of critically ill patients.[27] For subjective physical assessment, low muscularity was recorded for participants who displayed mild-moderate or severe muscle wasting using the SGA tool.

Statistical analysis

For this pilot study, a pragmatic sample size of 50 patients was chosen. SPSS, version 25 (IBP Corp., Armonk, NY, USA) was used for all analyses. Shapiro–Wilk tests were used to assess normality. Data are reported as *n* (%), mean (SD), or median and interquartile range (IQR). Missing data were not imputed. Differences in mean CT muscle area and bedside methods of assessing muscularity by sex and age (<65 years versus ≥65 years) were assessed using independent Student's *t* tests.[30] Pearson's correlation was used to assess the relationship between CT muscle area and bedside measures.

Cohen's kappa statistic (κ) was used to evaluate the agreement between muscularity status (normal or low) assessed by the bedside methods and CT image analysis. For all analyses, $P < 0.05$ was considered statistically significant.

Results

Participants

Of 1580 patients were screened, and of the 373 patients who had a CT scan including the L3 area, 323 patients were excluded, leaving 50 participants included in the primary study.[19] Of these, all participants had a subjective physical assessment, 41 (82%) had arm anthropometry and 26 (52%) had BIS data recorded. The CONSORT diagram is shown in Figure 1.

Participants were predominantly Caucasian, male [38 (76%)], young [<65 years old (33 (66%)] and admitted post-trauma injury [42 (84%)]. Participant characteristics for the entire cohort of 50 patients and for the subgroups with valid BIS and arm anthropometry measurements are shown in Table 1. The mean (SD) time from ICU admission to performing the bedside measurements was 33 (12) h, and that from CT scan to the bedside measurements was 26 (13) h. Mean values for CT muscle area, FFM, phase angle, mid-upper arm circumference, mid-arm muscle circumference; by age and sex are shown in Table 2. Mean (SD) fluid balance for the 24-h period before performing the bedside protocol was +1726 (1354) mL for the total cohort ($n = 31/50$), +1755 (1266) mL for the group with BIS measurements

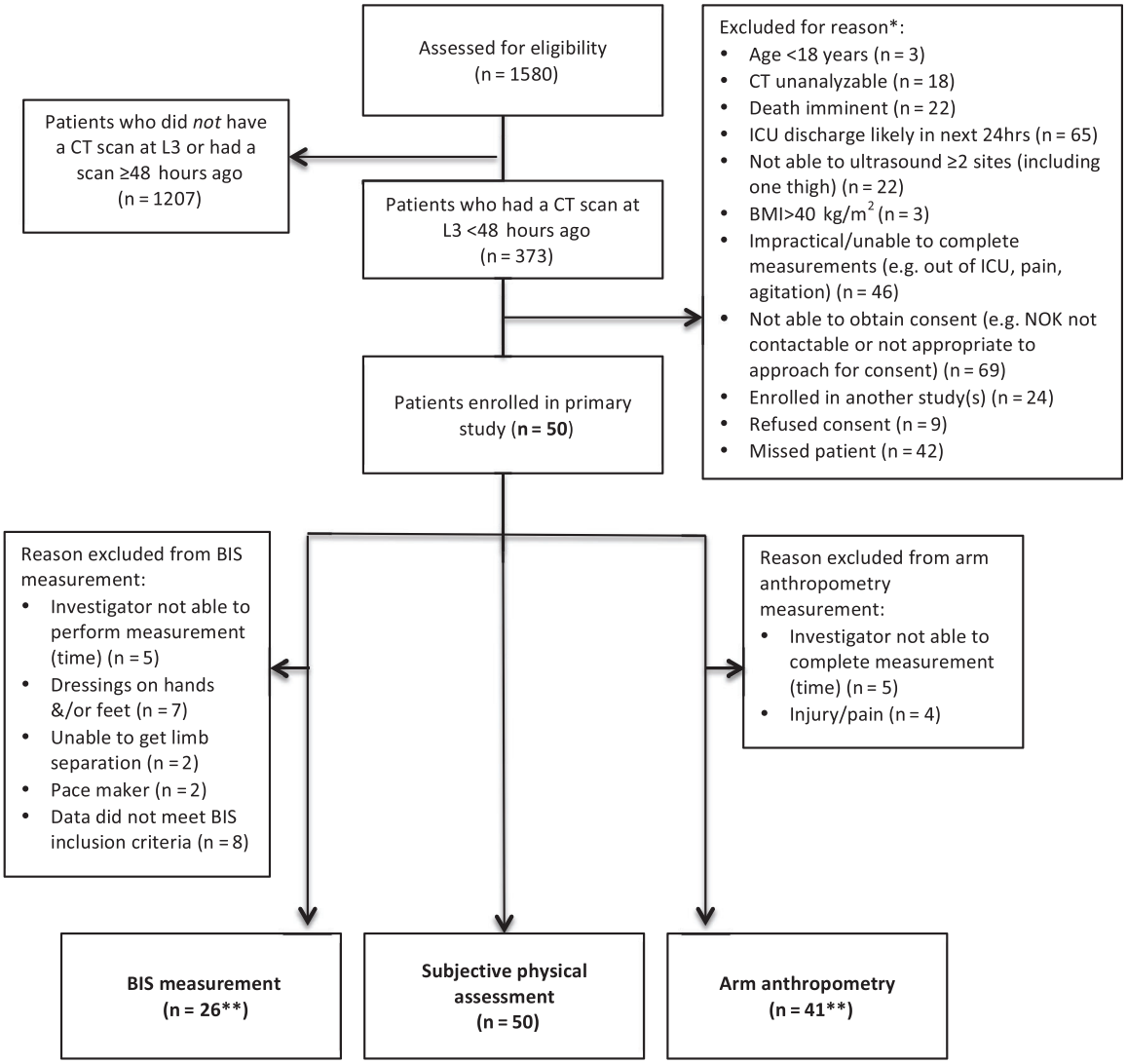

Figure 1 CONSORT diagram. BMI, body mass index; BIS, bioimpedance spectroscopy; CT, computed tomography; L3, third lumbar; NOK, next of kin. *Reasons for exclusion were based on primary study [19]. **Number of patients out of the 50 patients enrolled in the primary study [19] who had a valid measurement.

(n = 18/26) and +1748 (1248) mL for the group with arm anthropometry measurements (n = 26/41).

Correlation between fat-free mass and arm anthropometry and computed tomography muscle area

There were strong positive and significant correlations between CT muscle area and FFM-BIS (kg) (r = 0.801, P < 0.001) and FFM-Chamney (kg) (r = 0.807, P < 0.001), Figure 2. Mid-arm muscle circumference was more strongly correlated with CT muscle area than mid-upper arm circumference (r = 0.665, P < 0.001 versus r = 0.342, P = 0.029) (Figure 2).

Correlation between phase angle and computed tomography muscle area and density

Phase angle was significantly correlated with CT muscle area (r = 0.589, P < 0.001) and CT muscle density (r = 0.776, P < 0.001) (Figure 3).

Agreement between bedside and computed tomography assessment of muscularity status

In the group who had BIS measurements (n = 26), there were six (23%) participants who had low CT muscle area. Using FFM-BIS values, two (33%) participants were

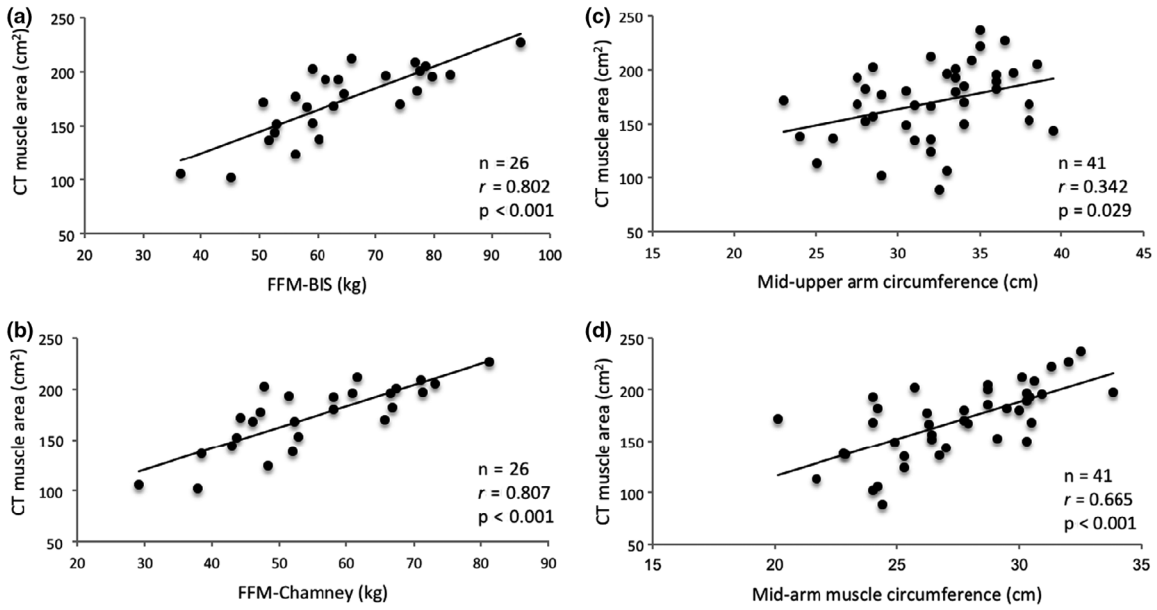

Figure 2 Correlation between computed tomography (CT) muscle area, fat-free mass (FFM)- Bioimpedance spectroscopy (BIS) (a), FFM-Chamney (b), mid-upper arm circumference (c), and mid-arm muscle circumference (d).

correctly classified as having low CT muscle area [$\kappa = 0.435$, 95% confidence interval (CI) = 0.016–0.854, $P = 0.007$]. Using FFM-Chamney values, all six (100%) participants who had low CT muscle area were detected ($\kappa = 0.723$, 95% CI = 0.441–1.00, $P < 0.0005$).

In the 41 participants who had arm anthropometry measurements, 13 (32%) had low CT muscle area. Anthropometry had a poor ability to classify participants with low CT muscle area, with four (31%) being correctly classified using mid-upper arm circumference ($\kappa = 0.060$, 95% CI = −0.249–0.309, $P = 0.698$) and five (38%) using mid arm muscle circumference ($\kappa = 0.137$, 95% CI = −0.178 to 0.452, $P = 0.378$).

In the total cohort ($n = 50$), 14 (28%) participants had low CT muscle area. Of these, four (28%) participants were correctly classified as having low CT muscle area using the physical assessment tool ($\kappa = 0.365$, 95% CI = 0.093–0.637 $P = 0.001$).

Discussion

In this exploratory prospective observational study, we report that BIS-derived FFM, adjusted using the Chamney model, which accounts for fluid overload, was significantly correlated with CT muscle area and had good agreement with muscularity status assessed by CT image analysis. Other bedside methods; FFM-BIS (i.e. unadjusted), arm anthropometry and subjective physical assessment, although correlated with CT muscle area, performed poorly in correctly classifying participant muscularity status. BIS-derived phase angle, which has been identified as a potential predictor of outcome in critically ill, had a stronger relationship to CT muscle *density* than to CT muscle area.

Recently, two prospective single-centre studies investigated how muscle mass derived from bioimpedance techniques relates to CT muscle area in critically ill adults. In both studies, Looijaard *et al.* [32] (Netherlands) and Kim *et al.* [33] (Korea) compared CT- and BIA-derived indices of muscularity (one using a multifrequency BIA device and the other using a single-frequency BIA device). In agreement with our data, both studies found significant correlations between muscularity assessed by the two methods. However, correlation coefficients do not identify the measurement error (agreement) between two techniques.[12] To do this, variables must be converted using prediction equations into comparable parameters, which in turn introduces additional assumptions. To assess the agreement between the two methods, the two studies converted CT muscle area (cm^2) into skeletal muscle mass (kg) using the Shen equation [10]. Both studies reported that BIA significantly overestimated SMM; with increasing disagreement at higher muscle mass. Kim *et al.* [33] reported a significant mean (SD) bias of 3.4 (5.6) kg and wide limits of agreements between CT and MF-BIA-derived muscle mass and Looijaard *et al.* [33] found significant differences between CT and SF-BIA-derived muscle mass values from all three equations used (mean biases 2.4–6.9 kg with wide limits of agreement) [32,33]. Because these types of analyses require the use of multiple muscle

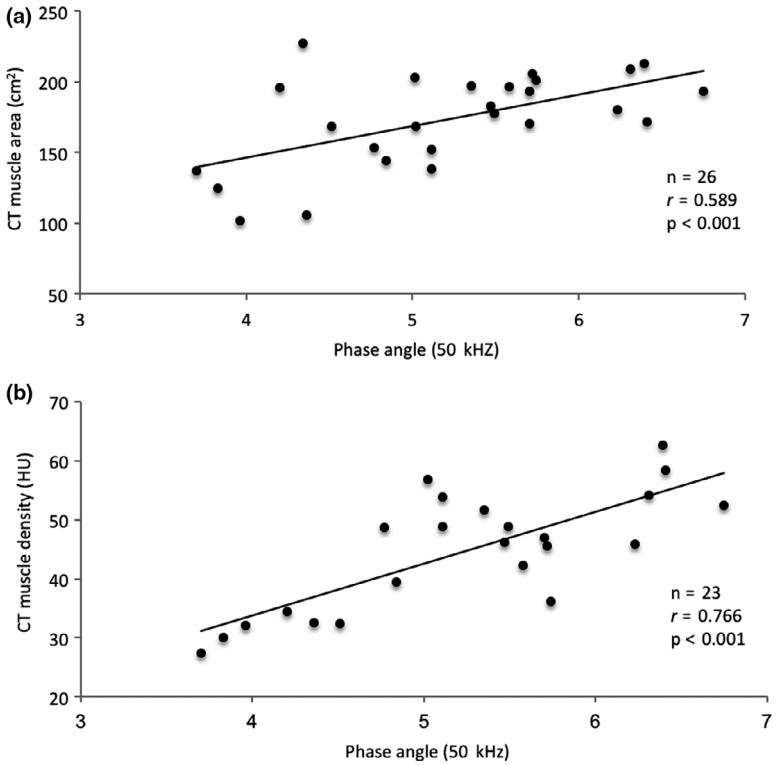

Figure 3 Relationship between phase angle and computed tomography (CT) muscle area (a) and CT muscle density (b).

mass equations, which in turn rely on a range of assumptions that to our knowledge have not been validated for the BIS technology we used, we elected not to undertake such explorations. However, the data from these studies and our study suggest that BIA-derived FFM estimates compared to CT muscle area in the ICU setting are variable and are influenced by hydration status, ethnicity and the equations used to derive FFM values, and so caution should be exercised when interpreting data.

Because unadjusted estimates of FFM in critical illness using bioimpedance technology may be confounded by fluid overload, raw data from bioimpedance devices (which are independent of weight), such as phase angle, are being increasingly explored in the ICU setting. Specifically, phase angle at ICU admission has been associated with increased survival in two prospective observational studies [13,34] and predicted live ICU discharge in another.[35] Although phase angle may be an important predictor of outcome in critically ill patients, the mechanisms for these findings are not entirely understood. Recently, the relationship between phase angle and muscularity has been explored and, in agreement with the findings of the present study, a moderate correlation between phase angle and CT muscle area was reported [32,35]. In the present study, we observed a stronger correlation between phase angle and CT muscle density compared to CT muscle area ($r = 0.776$ and $r = 0.589$, respectively) and these findings are consistent with those of Looijaard *et al.* ($r = 0.701$ versus $r = 0.542$).[32] These data fit with the theory that phase angle is reflective of cell membrane integrity and quality.[36] Further research is required to understand what phase angle threshold is predictive of poor outcome and what changes in phase angle over time correlate with nutritional changes that evolve with the illness course or resulting from nutritional interventions.

Finding a bedside assessment method to identify patients with lower than normal muscularity accurately is a key critical care nutrition research priority.[37] In the present study, the only bedside method that performed well in correctly classifying patients with low CT muscle area was FFM-Chamney values ($\kappa = 0.723$). These findings are in agreement with the study by Looijaard *et al.* [32] mentioned above, who reported that BIA (using the Talluri equation) had a good ability to identify patients with low CT muscle area (area under the curve: males 0.919; females 0.912).[32] These results highlight that bioimpedance technology may be useful to identify patients with low muscularity on ICU admission, although, as we have shown in the present study, accuracy is likely to be dependent on the equation used to derive FFM values and also on whether any adjustment

Table 1 Patient characteristics*

Characteristics	All patients (n = 50)	BIS (n = 26)	Arm anthropometry (n = 41)
Age years, mean (SD)	52 (20)	48 (18)	53 (19)
Age category, *n* (%)			
<65 years	33 (66)	20 (77)	27 (66)
≥65 years	17 (34)	6 (23)	14 (34)
Sex, *n* (%)			
Male	38 (76)	20 (77)	31 (76)
Female	12 (24)	6 (23)	10 (24)
APACHE II	12 (9–16)	12 (9–15)	12 (10–16)
APACHE III	45 (35–65)	43 (33–61)	44 (35–66)
Height m, mean (SD)	1.72 (0.09)	1.71 (0.08)	1.71 (0.08)
Weight kg, mean (SD)	82 (15)	84 (15)	83 (15)
BMI (kg m^{-2}), mean (SD)	28 (5)	28 (5)	28 (5)
Underweight, *n* (%)	1 (2)	0	1 (2)
Normal weight, *n* (%)	15 (30)	6 (23)	10 (24)
Overweight, *n* (%)	18 (36)	12 (46)	16 (39)
Obese, *n* (%)	16 (32)	8 (31)	14 (34)
Comorbidity index, mean (SD)	2 (2)	2 (2)	2 (2)
Admission category, *n* (%)			
Trauma	42 (84)	22 (84)	33 (81)
Medical	7 (14)	3 (12)	7 (17)
Surgical	1 (2)	1 (4)	1 (2)
Patients MV, *n* (%)	31 (62)	17 (65)	26 (63)
ICU LOS, days	5 (2–11)	5 (2–12)	6 (2–11)
Hospital LOS, days	16 (11–24)	15 (8–23)	15 (9–23)

APACHE, Acute Physiology and Chronic Health Evaluation; BMI, body mass index; ICU, intensive care unit; LOS, length of stay; MV, mechanically ventilated.

*Values are presented as the median (interquartile range) unless stated otherwise.

for hydration status is made. It is useful to remember that our fluid-adjusted FFM variable is calculated from ICW and ECW values, and is quite likely to be a more specific representation of the muscle compartment than the more broad FFM variable, which is based on a two-component conceptualisation of body composition. Thus, it is not surprising that this fluid-adjusted FFM variable is more closely aligned with the CT muscle data. Future studies are required to understand how low muscularity assessed by bioimpedance technology relates to clinical and functional outcomes in critically ill adults. Accounting for fluid status may be an important consideration when assessing the ability of bioimpedance technology to predict outcome, as has been shown in renal patients receiving dialysis, with BIS-derived normally hydrated lean tissue (i.e. FFM-Chamney values in the present study) and fluid overload (assessed using the Chamney model) being associated with mortality [38,39].

In the present study, we found that arm anthropometry and subjective physical assessment did not perform well in identifying those patients with low CT muscle area. The accuracy of using mid-upper arm circumference to measure and track changes in muscularity in critically ill patients has been questioned before. In a study by Campbell *et al.* [40], nine patients with multiorgan failure were studied, and muscle thickness (via ultrasound) and mid-upper arm circumference were measured every 1–4 days early in the ICU admission. Using ultrasound, all patients showed a significant, consistent decrease in muscle thickness over time (a finding which has been replicated in subsequent studies).[41] By contrast, the arm circumference measurements showed no consistent pattern of change.[40] It was hypothesised that oedema most likely influenced arm measurements, thus rendering them of low utility.

Similarly, the ability of the SGA tool to identify individuals with low muscularity in critically ill patients has also been challenged. Nutrition assessment using the SGA tool was undertaken in a study of critically ill respiratory patients who had a CT scan at the L3 area, finding that 63% of patients with low CT muscle area were misclassified as normally nourished (where the CT scan and SGA were performed within 3 days of each other).[42] These findings are similar to the present study, where 72% of participants with low CT muscle area were not detected by subjective physical assessment using the SGA tool.

The present study has strengths and limitations. We used standardised methodology to identify and exclude participants with extreme and unphysiological bioimpedance variables (e.g. as a result of fluid overload). These findings contribute to the literature with respect to understanding the capabilities of BIS to provide more specific estimates of muscle, when accounting for patients with extreme fluid overload and/or potential measurement errors (e.g. inadequate limb separation, interference with other bedside machinery). They also importantly highlight that lean tissue depletion may be masked using standard bioimpedance techniques (without adjusting for fluid status). BIS-derived measurements of lean tissue, when adjusted for fluid status using approaches such as Chamney modelling, also show potential for use in clinical practice to use body composition-based approaches for the diagnosis of malnutrition, such as the GLIM. Indeed, this approach could improve on the diagnosis of malnutrition in critical illness and other populations with fluid-overload compared to simpler single-frequency-

Table 2 Values for computed tomography (CT) muscle area and each bedside method by sex and age category*

Variable	*n*	All patients	*n*	Male	*n*	Female	*P* value	*n*	Younger (<65 years)	*n*	Older (≥65 years)	*P* value
(A) CT and BIS												
CT muscle area, cm^2	26	173.4 (33.0)	20	185.9 (23.9)	6	132.9 (26.6)	0.001	20	184.2 (23.1)	6	137.3 (37.1)	0.001
FFM-BIS, kg	26	64.2 (13.2)	20	68.1 (11.9)	6	51.3 (8.8)	0.004	20	68.4 (11.5)	6	50.2 (8.2)	0.001
FFM-Chamney, kg	26	55.2 (12.8)	20	58.6 (11.8)	6	43.8 (9.2)	0.010	20	59.5 (10.8)	6	40.8 (7.2)	0.001
Phase angle, 50 kHz	26	5.3 (0.8)	20	5.5 (0.8)	6	4.5 (0.5)	0.008	20	5.6 (0.7)	6	4.4 (0.6)	0.001
(B) CT and arm anthropometry												
CT muscle area, cm^2	41	170.0 (35.0)	31	182.8 (26.4)	10	130.1 (27.7)	0.001	27	183.1 (28.1)	14	144.7 (28.1)	0.001
Mid-upper arm circumference, cm	41	32 (4)	31	32 (4)	10	32 (4)	0.897	27	33 (5)	14	31 (3)	0.267
Mid-arm muscle circumference, cm	41	27 (3)	31	28 (3)	10	25 (2)	0.009	27	28 (3)	14	26 (2)	0.089

BIS, bioimpedance spectroscopy; CT, computed tomography.
*Values are presented as the mean (standard deviation).

derived FFM cutpoints. Furthermore, the bioimpedance FFM-index cut points provided in the GLIM criteria were derived from a 50-kHz FFM equation developed from Swiss Caucasian population data, and their application to bioimpedance data derived from other devices, as well as in other populations, is inherently limited, and not likely to effectively identify all patients with low muscularity. Despite these limitations, our findings highlight the potential value that a simple BIS-derived fluid-adjusted measure of lean tissue can have with respect to the identification of low muscularity and malnutrition. This is important for identifying those individuals who may need targeted interventions, and also because weight-based indicators, simple anthropometry and subjective physical assessment may not be sensitive enough to detect malnutrition.[42] Limitations of the study include the modest sample size and generalisability of the results, with the majority of participants being young and previously well trauma patients (as a result of a requirement for a CT at L3 to enter the study). Additionally, an estimated weight was used for input into the BIS device (with anticipated challenges with obtaining an accurate weight on ICU admission, e.g. as a result of drain tubes, dressings, etc.). It also remains unclear whether there is a linear relationship between CT muscle area at the L3 region and whole-body muscularity in critically ill patients.

Conclusions

In this pilot study, a unique BIS-derived FFM variable using an equation that accounts for fluid overload was significantly correlated to CT muscle area and was able to correctly classify all the participants with low CT muscle area at ICU admission. Arm anthropometry and subjective physical assessment were not able to readily detect patients with low CT muscle area. Phase angle had a stronger relationship to CT muscle density compared to the muscle area. Future studies should investigate how low muscularity assessed by BIS (ideally using an equation or method to account for fluid overload) relates to clinical and functional outcomes in critically ill patients.

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Conflicts of interest, source of funding and authorship

In the past, CPE has received small monetary support and loaner bioimpedance devices from Bodystat LTD, ImpediMed and InBody. All other authors declare that they have no potential conflicts of interest. KJL was supported by an Australian Government Research Training Scholarship. KJL, CPE, ACT, AF and SJK contributed to the conception and design of the research. KJL, CPE, GSG and SJK contributed to the acquisition and analysis of the data. KJL, CPE and SJK contributed to the interpretation of the data. KJL and SJK drafted the manuscript. All authors critically revised the manuscript, agree to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final version of the manuscript submitted for publication.

Transparency declaration

The lead author affirms that this manuscript is an honest accurate and transparent account of the study being

reported and is compliant with the STROBE guidelines. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned (which was approved by the research and ethics committees at The Alfred hospital and La Trobe University) have been explained.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Checklist S1. Checklist of items that should be included in reports of cross-sectional studies.

4.4 Conclusion

This chapter describes pilot data which found that a novel FFM variable using an adjustment algorithm for overhydration was correlated with CT muscle CSA and had good agreement with muscularity status assessed by CT image analysis. Future studies should investigate how low muscularity assessed by BIS (ideally using an adjustment for overhydration) relates to clinical and functional outcomes in critically ill patients.

The study presented in this chapter also found that arm anthropometry and subjective physical assessment were unable to reliably detect patients with low muscularity at ICU admission. With these methods still used in clinical practice internationally, it is important that clinicians are aware of the limitations with these approaches to assessing muscle mass in critically ill patients, particularly at ICU admission.

The next chapter moves direction to explore the relationship between energy and protein delivery and changes in skeletal muscle mass in critical illness.

Chapter 5

What is the association between energy and protein delivery and skeletal muscle mass changes in critical illness?

A SYSTEMATIC LITERATURE REVIEW

5.1 Declaration of authorship

Student's declaration:

The nature and extent of contributions to Chapter 5 of this thesis are as follows:

Name	Nature of contribution	Contribution
Kate Lambell	Study concept and design (search strategy), screening of articles, quality assessment and data extraction, data analysis and interpretation, manuscript preparation and revision for publication	85%
Susannah King	Quality assessment and data extraction, data interpretation, revision of manuscript	5%
Adrienne Forsyth	Quality assessment and data extraction, data interpretation, revision of manuscript	5%
Audrey Tierney	Data interpretation, revision of manuscript	5%

Supervisor's declaration:

I hereby certify that the declaration above is a correct reflection of the extent and nature of contributions made toward Chapter 5 of this thesis by the student and all listed co-authors.

Name of supervisor	Signature
Susannah King	

5.2 Introduction

Critically ill patients experience significant and rapid loss of skeletal muscle mass, which has been associated with adverse clinical outcomes (as described in detail in section 1.3). The aetiology of muscle wasting is multifactorial and nutrition delivery may play a role[26,145,146]. This chapter describes a systematic literature review undertaken to investigate the association between energy and protein delivery and skeletal muscle mass changes in critical illness. The work in this chapter relates to thesis objective 5:

- To investigate the association between energy and protein delivery and skeletal muscle changes in critical illness.

The results for this chapter are presented in the form of a manuscript published in the *Journal of Parenteral and Enteral Nutrition (Impact Factor 4.016), the citation is as follows:*

> Lambell KJ, King SJ, Forsyth AK, Tierney AC. Association of energy and protein delivery on skeletal muscle mass changes in critically ill adults: a systematic review. *JPEN*. 2018 Sep;42(7):1112-22. DOI: 10.1002/jpen.1151

As at 17th July, 2021 the publication has been cited 23 times. The findings were also accepted for an oral presentation at the Australasian Society for Parenteral and Enteral Nutrition (AuSPEN) annual scientific meeting in Melbourne, 2016.

After the manuscript, the search strategy is displayed as a supplementary table. The original literature search was run on the 1st March, 2016. Due to the extended time between this search and thesis submission, a re-run of the systematic literature search

was performed and the results, discussion, and implications are displayed at the end of the chapter.

5.3 Manuscript

"Association of Energy and Protein Delivery on Skeletal Muscle Mass Changes in Critically Ill Adults: A Systematic Review"

Review

aspen LEADING THE SCIENCE AND PRACTICE OF CLINICAL NUTRITION
American Society for Parenteral and Enteral Nutrition

Association of Energy and Protein Delivery on Skeletal Muscle Mass Changes in Critically Ill Adults: A Systematic Review

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Abstract

Critically ill patients experience significant and rapid loss of skeletal muscle mass, which has been associated with negative clinical outcomes. The aetiology of muscle wasting is multifactorial and nutrition delivery may play a role. A systematic literature review was conducted to examine the association of energy and/or protein provision on changes in skeletal muscle mass in critically ill patients. Key databases were searched up until March 2016 to identify studies that measured skeletal muscle mass and/or total body protein (TBP) at 2 or more time points during acute critical illness (up to 2 weeks after an intensive care unit [ICU] stay). Studies were included if there was documentation of participant energy balance or mean energy delivered to participants during the time period between body composition measurements. Six studies met inclusion criteria. A variety of methods were used to assess skeletal muscle mass or TBP. Participants in included studies experienced differing levels of muscle loss (0%–22.5%) during the first 2 weeks of ICU admission. No association between energy and protein delivery and changes in skeletal muscle mass were observed. This review highlights that there is currently limited high-quality evidence to clearly define the association between energy and/or protein delivery and skeletal muscle mass changes in acute critical illness. Future studies in this area should be adequately powered, account for all potential confounding factors to changes in skeletal muscle mass, and detail all sources and quantities of energy and protein delivered to participants. (*JPEN J Parenter Enteral Nutr.* 2018;42:1112–1122)

Keywords

critical illness; intensive care; nutrition therapy; skeletal muscle; systematic review

Introduction

Skeletal muscle is the most abundant tissue in the human body and comprises the majority of lean body mass.[1] In addition to its functional role in movement, skeletal muscle plays an important role in the body's ability to respond to illness by acting as a crucial reservoir of amino acids to aid in cell repair when needed.[2,3] It also has important metabolic and immunologic functions by storing glucose and triglycerides, supplying a substrate for gluconeogenesis, and acting as a secretory tissue, releasing numerous myokines.[1,4]

In stressed states, such as critical illness, there is a need for accelerated synthesis of acute-phase proteins in the liver and proteins involved in immune function and wound healing, which are necessary for recovery from illness.[5] The demands for precursor amino acids for the synthesis of these proteins results in muscle protein breakdown and is confirmed by reports of rapid and significant loss of skeletal muscle and whole body protein turnover up to 3% per day in the acute stages of illness.[6-13] Low skeletal muscle mass and loss of lean tissue in chronic and acute illness have been associated with negative clinical outcomes, including increased incidence of infections,[14,15] muscle weakness,[7] length of stay,[11,16] and mortality.[17-19] These, along with the immunologic and metabolic functions of skeletal muscle, emphasize the importance of preserving muscle mass, particularly in acute illness.[1,4]

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Although the maintenance of skeletal muscle mass in the critically ill is important for recovery, the causes and mechanisms for muscle depletion remain poorly understood. It is thought to be a multifactorial process involving complex neuroendocrine responses, disease severity, infection, insulin resistance, corticosteroid use, nerve and neuromuscular junction changes, polyneuropathy, immobility, and nutrition (energy and protein) deficit.[20-22]

A greater understanding of the relationship between energy and protein delivery on skeletal muscle mass changes in critical illness may contribute to optimal targeting of nutrition therapy for muscle maintenance and, consequently, improved rehabilitation of critically ill survivors. The aim of this study was to review available evidence to ascertain if there is an association between energy and/or protein delivery and changes in skeletal muscle mass during acute critical illness.

Methods

A systematic review of the literature was performed following the preferred reporting items for systematic reviews and meta-analyses (PRISMA) criteria for conducting and reporting systematic reviews.[23] Methods and eligibility criteria were prespecified and documented in a protocol registered on PROSPERO, the international prospective register of systematic reviews (registration number CRD42016035575) and also summarized below.

Criteria for the inclusion of literature were critically ill adult participants aged ≥18 years admitted to an intensive care unit (ICU), where skeletal muscle mass and/or total body protein (TBP), a direct measurement of skeletal muscle protein and visceral protein, were measured on at least 2 occasions during acute critical illness (up to 2 weeks after ICU admission). Documentation of mean energy, with or without protein, delivered to participants in the time period between the measurement of skeletal muscle mass and/or TBP was also required for eligibility. The search strategy was modified after the protocol was published to also include studies reporting energy balance (in the absence of reporting intake and expenditure/requirement data), on the basis that energy balance data derived from either direct reporting or by calculation from each article were valuable for answering the review question.

Studies that measured skeletal muscle mass using single-frequency bioimpedance analysis, mid-arm muscle circumference, or subjective physical assessment were excluded because they have accuracy limitations in the ICU population.[10,24,25] Because this review focused on changes in muscle volume, studies reporting nitrogen balance and changes at a cellular level, such as molecular pathways, muscle quality, and muscle fibers, were excluded.

The outcome of interest was change in skeletal muscle mass measured via dual-energy x-ray absorptiometry (kg); computed tomography (CT; cm^2, cm^3); ultrasound of the forearm, mid–upper arm, and/or quadriceps muscle layer thickness (cm) or quadriceps (rectus femoris) cross-sectional area (cm^2), and/or TBP via prompt γ in vivo neutron activation analysis (kg). TBP is calculated from total body nitrogen and can be further subdivided into skeletal muscle protein and visceral protein.[26] As the included studies reported TBP, for the purpose of this review we report TBP as an associated measure of skeletal muscle mass.

CONCEPT A : Population (Critically ill patients) AND

CONCEPT B : Intervention (Method to measure body composition) AND

CONCEPT C : Outcome (Skeletal Muscle Mass and/or Total Body Protein)

Figure 1. Search strategy by combining key concepts

No restrictions were imposed on study design due to the limited number of randomized controlled trials. Only fully published articles were included. The review did not include non-English publications and non-primary studies (case studies, narrative reviews, editorials, letters, commentaries, guidelines, or grey literature).

A systematic literature search was conducted using 4 different databases (Ovid MEDLINE, EMBASE, CINAHL and the Cochrane Central Register of Controlled Trials). Databases were searched from 1946 to March 1, 2016. The search strategy (Figure 1) was wide-ranging, using 3 key concepts to give the best opportunity to include all relevant literature. To maximize the possibility of identifying all relevant studies, both free text terms and broad search terms (i.e., MeSH in PubMed and CINAHL Headings in CINAHL) were used. Examples include: critical care, critical illness; multiple organ failure; ICUs; ultrasonography; tomography, x-ray computed; magnetic resonance imaging; whole body imaging; absorptiometry, photon; electric impedance; neutron activation analysis; muscle, skeletal; fat free mass; body composition; muscular atrophy and sarcopenia. The full set of search terms is available in Supplement Table S1.

To ensure all relevant publications were identified, references and citations of included articles along with indices of the 2 key online journals in this area, *Journal of Parenteral and Enteral Nutrition* and *Critical Care* (2014–2016), were hand searched.

The primary researcher (KJL) assessed eligibility of each study by reviewing the title and abstracts. Full texts were obtained and where required, authors were contacted if there was information lacking. Two senior researchers (AKF and SJK) each assessed eligibility of 25 randomly selected articles from the search database. The findings were compared with those of the principal author and any ambiguous articles were discussed within the study team and eligibility was determined by consensus.

Data from the eligible studies were extracted and refined during the process of data extraction. Where appropriate, authors were contacted to request or confirm extracted data. The American Dietetic Association (ADA) Quality Criteria Checklist for Primary Research,[27] a tool recommended for both randomized and non-randomized nutrition studies, was used to assess the quality of included studies based on the primary aims and objectives of each study. This allowed for study quality to be rated as positive, neutral, or negative according to 10 questions assessing risk of bias. This tool was chosen in preference over the Downs and Black quality instrument (which was mentioned but not changed in the registered protocol) after consensus among investigators that it provided a more targeted approach to critically appraising nutrition-related studies. The principal author (KJL) and senior researchers (AKF and SJK) independently assessed the quality of the included studies.

International guidelines recommend critically ill patients receive a range of 20–30 kcal/kilogram (kg) body weight (lower range in the initial phase of critical illness and increased during the anabolic recovery phase) and 1.2–2 g protein/kg per day.[28,29] To compare the nutrition provision in each study against these guidelines, energy and protein delivery were represented as mean kcal and grams of protein delivered/kg per day during the study period. Weight was taken as either the estimated mean participant weight reported in the study (actual or obesity-adjusted body weight) or the measured weight on day 10, which has been reported to be the closest to pre-illness weight.[30,31]

Results

The search strategy yielded 2092 publications of which 424 were duplicates, leaving 1668 unique publications for screening based on title and abstract (Figure 2). A total of 1653 articles were excluded for various reasons, leaving 15 articles for examination. Four studies using single-frequency bioimpedance analysis and 5 studies with duplicate data were excluded,[30-34] leaving 6 studies meeting the inclusion criteria.[8,9,12,13,35,36] There were no discrepancies between researchers in terms of eligibility of the studies, data extraction, or study quality.

Table 1 outlines the characteristics of the included studies. Two studies were given a positive rating using the ADA checklist.[8,35] Four studies were given a neutral rating because they showed weaknesses in design, most commonly, not accounting for potential confounders of changes in skeletal muscle mass (i.e. organ failure). No study received a negative rating.

Almost all studies had a small sample size (n = 15–119), and only 1 study was powered to detect a change in skeletal muscle volume.[8] Two studies did not aim to observe the impact of nutrition on skeletal muscle changes with time, which resulted in limited data on all sources of nutrition delivered to participants.[8,36]

Participant characteristics in the included studies showed heterogeneity, reflective of usual ICU populations.[37] This was most commonly described using the Acute Physiology and Chronic Health Evaluation (APACHE II) score to report severity of illness on ICU admission,[38] which varied widely (17–30), as did participant age (44–67 years), sex (40%–67% male) and reason for admission.

A range of body composition methods and anatomic sites was used to describe changes in skeletal muscle mass or TBP across the acute stage of illness. Specifically, in a specialist body composition unit, Uehara et al measured TBP via in vivo neutron activation analysis.[36] Two studies, both substudies of randomized controlled trials, assessed skeletal muscle mass via CT image analysis.[9,13] Braunschweig et al[9] used CT images (containing the third lumbar area) performed for diagnostic purposes to quantify abdominal cross-sectional area (CSA). Casaer et al[13] included patients who were scheduled for repeat follow-up CT scan of the brain within 48 hours after admission and after 1 week for clinical purposes. Following the brain scan, the patients were then repositioned to quantify skeletal muscle cross-sectional volume at the abdomen and midfemoral area. The remaining 3 studies used ultrasound to measure either muscle layer thickness of the forearm, mid–upper arm and/or quadriceps or quadriceps (rectus femoris) CSA.[8,12,35]

In 4 studies, skeletal muscle mass was first measured on mean ICU day 1-day 2 with sequential measurement(s) occurring at various points up to 7–10 days after ICU admission.[8,12,13,35] One study reported the mean period between measurements being 10 days but did not state the mean ICU day in which the 2 measurements were taken.[9] The remaining study measured TBP until around 2 weeks after admission (with the first measurement at mean ICU day 3 when hemodynamic stability was achieved).[36] Loss of skeletal muscle and TBP varied greatly across the studies, from no change to a loss of 22.5% during the study period.

Energy: Sources, Expenditure, Delivery, and Impact On Skeletal Muscle Mass Changes

Participants were delivered energy via enteral and parenteral nutrition in all studies. One study also collected energy intake data from oral diet.[9] Administration of non-nutrition sources of energy was poorly described and attempts were made to contact authors for any missing data. Only 2 studies reported energy delivery from both intravenous dextrose and propofol (either in their article or via response from the author).[9,35]

Calculation of participant energy requirements varied across the studies. Two studies measured resting energy expenditure via indirect calorimetry, with 1 study

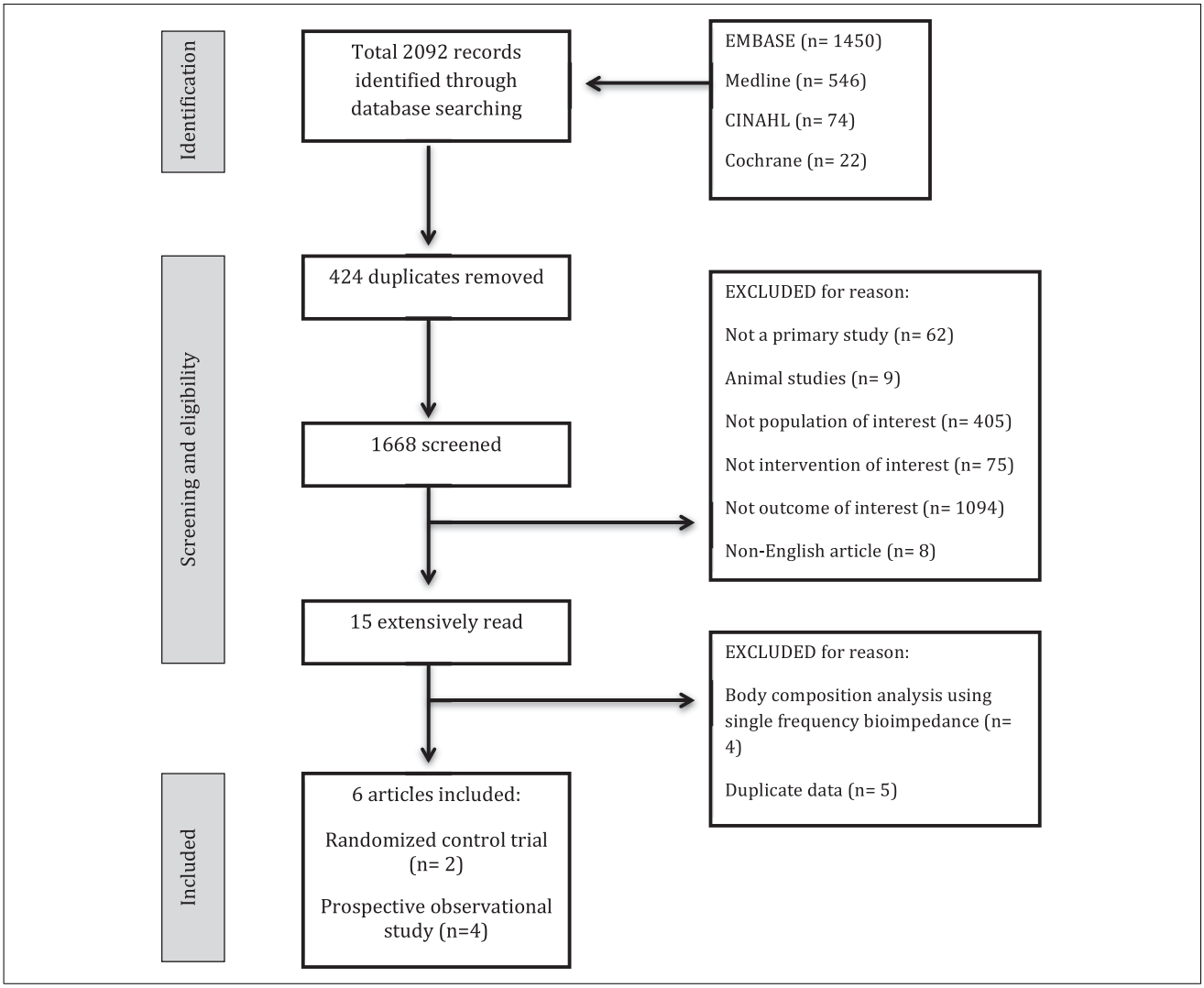

Figure 2. Flow diagram of search strategy

measuring this over a 24-hour period[12] and the other study twice daily until day 10 of ICU stay.[36] In 2 studies, energy delivery was targeted using 25[35] and 30[9] kcal/kg body weight or obesity-adjusted body weight. In the remaining study, the calculation of energy requirements was not stated or recorded.[8]

Mean daily energy intake varied greatly across studies (870 kcal–1840 kcal/day). When expressed as kcal/kg, energy provision ranged from 10.7 to 26.2 kcal/kg/day. Energy adequacy (energy delivery vs measured or estimated energy needs) was reported in or calculated from 4 studies.[9,12,35,36] In these studies mean energy delivered to participants ranged from 40% to 100% of prescribed requirements.

At an individual study level, Braunschweig et al[9] found that percentage of estimated energy requirements received was the only significant predictor of percentage change in skeletal muscle CSA loss per day across the study period ($\beta = 0.022$, $P = 0.03$).

Conversely, Reid et al[12] and Casaer et al[13] reported that increased energy provision was not associated with maintenance of muscle mass. When observing results for all studies there was no consistency between mean energy delivery and percentage loss of skeletal muscle mass or TBP.

Protein Delivery and Impact on Skeletal Muscle Mass Changes

Protein provision was poorly described, with only 3 studies reporting protein delivery.[9,35,39] In a fourth study, we were able to calculate this from published data and additional data provided by the author.[36] Protein delivery ranged from 0.6 g to 1.3 g/kg adjusted ideal body weight per day. Compared with current guidelines,[28,29] it is possible that

Table 1. Characteristics of Included Studies.

First author, year, country	Partici-pants, n	Participant demographics[d]	Intervention, study design, and aim(s)	Mean energy delivered (kcal/kg/day)[e]	Mean protein delivered (g/kg/day)[e]	Outcome measure and time between measurements	Results: % muscle loss during study period	Quality rating (ADA)	Comments
Braunschweig et al., 2014[9] United States	n = 33	Heterogeneous group Age 60 ± 16, Male sex 61%, BMI 28 ± 6, APACHE II 26 ± 7	EN, PN, and oral intake: Prospective observational study to 1) determine if CT scans completed for diagnostic purposes could be exploited to measure changes in abdominal skeletal muscle and fat depots, and 2) assess association between amount of estimated energy and protein needs received and changes in these depots	10.7	0.6	Quantitative CT: abdominal skeletal muscle CSA (cm^2) Measurements: mean 10 ± 5 days between 2 scans	Abdominal muscle CSA = decreased by 6.26% Loss averaged 0.49% per day, trended significant in both men ($P = 0.07$) and women ($P = 0.09$) Only predictor of change in muscle mass was percentage of estimated energy received ($P = 0.03$)	Neutral	Energy expenditure not measured
Casaer et al, 2013[13] Belgium	n = 15 Early PN; n = 10 Late PN; n = 5	Neurosurgical Early PN: Age 44 ± 16, Male sex 40%, BMI 24 (22–27), APACHE II 28 (26– 32); Late PN: Age 50 ± 16, Male sex 40%, BMI 25 (23–26), APACHE II 30 (24–30)	EN and PN: Substudy of a prospective RCT to document impact of early PN (when EN inadequate) vs late PN on muscle and adipose tissue volume and composition in small group of participants enrolled in larger RCT (EPaNIC trial)	Early PN 20.5; Late PN 14.5[g]	n/a	Quantitative CT: femoral and abdominal skeletal muscle CSV (cm^3) Measurements: median ICU day 2 (2–3) and day 9 (8–10)	Femoral muscle CSV = substantial loss in both groups, 6.9% ± 1.7%. Difference in percentage loss between early and late PN not significant ($P = 0.60$) Abdominal muscle CSV = no statistical evidence of change in either group	Neutral	Energy expenditure not measured Six matched healthy controls

(*continued*)

Table 1. (continued).

First author, year, country	Partici-pants, n	Participant demographics[d]	Intervention, study design, and aim(s)	Mean energy delivered (kcal/kg/day)[e]	Mean protein delivered (g/kg/day)[e]	Outcome measure and time between measurements	Results: % muscle loss during study period	Quality rating (ADA)	Comments
Ferrie et al, 2015[35] Australia	n = 119 Low protein: n = 60 High protein: n = 59	Heterogeneous group (largely surgical) Low protein: Age 65 (49–70), Male sex 60%, BMI 28, APACHE II 24 ± 8; High protein: Age 67 (55–74), Male sex 63%, BMI 26, APACHE II 26 ± 9	EN and PN: Prospective RCT to use quantitative and qualitative measures to compare standard intake of protein (0.8g/kg) with guideline recommendations (1.2g/kg) in critically ill patients requiring PN while controlling for energy intake	Low protein 24.9 ± 4.2; High protein 23.1 ± 3.9	Low protein 0.9 ± 0.2; High protein 1.1 ± 0.2	US (forearm, bicep, and thigh): muscle thickness (cm) and rectus femoris CSA (cm^2) Measurements: study day 0 (mean ICU day 1), 3, and 7	Muscle thickness (sum of 3 sites) = increased losses in low protein group (7.6%) vs high protein group (1.2%) at day 7 ($P = 0.02$) Rectus femoris CSA = decreased by 7.9% at day 7 in low protein group, no reported loss in high protein group[f] Significance between groups disappeared when imputed values used for missing data	Positive	Energy expenditure not measured Large amount of missing ultrasound data (39%)
Puthucheary et al, 2013[8] United Kingdom	n = 63	Heterogeneous Age 55(50–59)[c], Male sex 59%, BMI n/a, APACHE II 24 (22–25)	EN and PN: prospective observational study to characterize and evaluate time course and pathophysiology of acute muscle loss	≈15.8 (14.3–17.3)	0.6 (0.5–0.8)	US (quadriceps): rectus femoris CSA (cm^2) Measurements: day 1 (ICU), 3, 7, and 10	Rectus femoris CSA = decreased by 17.7% during 10 days ($P < 0.001$) Higher protein intake in first week associated with greater muscle loss	Positive	Energy expenditure not measured Powered to detect 10% reduction in rectus femoris CSA. Multicenter study

(*continued*)

Table 1. (continued).

First author, year, country	Partici-pants, n	Participant demographics[d]	Intervention, study design, and aim(s)	Mean energy delivered (kcal/kg/day)[e]	Mean protein delivered (g/kg/day)[e]	Outcome measure and time between measurements	Results: % muscle loss during study period	Quality rating (ADA)	Comments
Reid et al, 2004[12] United Kingdom	n = 50	Heterogeneous Age 56 (19–79), Male sex 52%, BMI n/a, APACHE II 17 (2–43)	EN and PN: prospective observational study to determine whether 1) ultrasound could identify and quantify muscle wasting in heterogeneous ICU population, and 2) relationship could be identified between rates of wasting and energy balance	n/a	n/a	US (forearm, bicep and thigh): muscle thickness (cm) Measurements: ICU day 1 and 1- to 3-day intervals for median of 7 days	Muscle thickness (sum of 3 sites) = decreased by 22.5%[g] with median loss of 1.6% per day in 48/50 participants Muscle thickness increased in 2 participants (1.1% and 0.6% per day) Energy balance made no difference to rate of wasting ($P = 0.508$)	Neutral	24-hour energy expenditure was measured in n = 24 Median daily energy balance was -8 kcal/kg Significant underfeeding and overfeeding according to measured energy expenditure
Uehara et al, 1999[36] New Zealand	N = 24 Sepsis n = 12 Trauma n = 12	Sepsis and Trauma Sepsis: Age 67 (25–84), Male sex 67%, BMI n/a, APACHE II 23 (15–34) Trauma: Age 34 (18–54), Male sex 75%, BMI n/a, APACHE II n/a	EN and PN: prospective observational study to obtain accurate values for components of energy expenditure in critically ill	Sepsis 26.2[f] Trauma 23.2[f]	Sepsis 1.1[f] Trauma 1.3[f]	IVNAA: TBP (g) Measurements: study day 0 (mean ICU day 3), 5, and 10 days later	TBP = decreased by 9.1% in the sepsis group and 12.0% in trauma group at day 10	Neutral	Mean energy delivered ≈100% of measured resting energy expenditure for both groups

ADA, American Dietetic Association; APACHE II, Acute Physiology and Chronic Health Evaluation; CSA, cross sectional area; CSV, cross sectional volume; CT, computed tomography; EN, enteral nutrition; IVNAA, in vivo neutron activation analysis; PN, parenteral nutrition; TBP, total body protein; US, ultrasound.
[a]Data presented as median (interquartile range).
[b]mean ± standard deviation.
[c]mean (95% CI).
[d]Data rounded to nearest whole number.
[e]Weight reported as mean participant weight (actual or obesity-adjusted) or measured weight on day 10.
[f]Additional data provided by author.
[g]Data extrapolated from figure presented in paper.

more than half of the participants in the included studies had inadequate protein provision.

Two studies investigated the association of protein delivery on measures of skeletal muscle assessed by ultrasound.[8,35] In Ferrie et al's randomized controlled trial comparing standard intake of amino acids with guideline recommendations, they delivered 0.9 g protein/kg per day in the standard group vs 1.1 g/kg per day in the intervention group while controlling for energy delivery. They observed a significant difference in ultrasound-measured muscle thickness at 3 sites (quadriceps, forearm, and bicep) at day 7 with muscle thickness of the lower protein group decreasing by 7.6% vs 1.2% in the higher protein group ($P = 0.02$). Additionally, there were no reported losses of rectus femoris CSA in the higher protein group over 7 days and 7.9% loss in the lower protein group (data provided by author). However, missing variables occurred for ultrasound measurements in approximately one-third of patients, which limits the strength of these findings. When observing the intention-to-treat analysis comparing outcomes based on imputed values, significance for these 2 parameters disappeared.[40] Puthucheary et al[8] observed significant loss of rectus femoris CSA during the first 10 days of ICU admission (17.7%) and reported an incidental finding that reduction in muscle volume was negatively associated with total protein delivery. Across all included studies there was no clear association between mean delivered grams of protein/kg per day vs percentage loss of skeletal muscle mass or TBP.

Potential Confounding Factors for Skeletal Muscle Mass Changes

The administration of medications (insulin, neuromuscular blocking agents, glucocorticoids, norepinephrine, aminoglycosides, inotropes, and/or steroids) was poorly described in all studies. No study reported association between medication delivery and changes in skeletal muscle mass. No study reported on participant physical activity or inactivity (i.e., days resting in bed or time to stand). Puthucheary et al[8] investigated the association of organ failure on skeletal muscle volume changes, reporting that an increasing organ failure score correlated with greater change in rectus femoris CSA ($P < .001$).

The influence of gender on observed changes in skeletal muscle mass was only reported in the study by Braunschweig et al.[9] In this study, women had a greater loss of muscle (11%) compared with men (4%) during the study period.

Reid et al[12] was the only group to examine level of muscularity on admission and rate of muscle loss. When they compared the 24 participants with the thickest muscles to the 24 participants with the thinnest muscles, they found that those with the greatest amount of muscle at the start lost significantly more muscle thickness than those with thinner muscles at baseline ($P < .001$).

Discussion

The aim of this systematic review was to investigate the association between energy and protein delivery and skeletal muscle mass changes in critical illness. The included studies used a variety of methods (CT, ultrasound, and in vivo neutron activation analysis) and sites (biceps, thigh, forearm, abdomen, and whole body) to observe changes in skeletal muscle during the first 2 weeks of ICU admission. The degree of muscle loss varied considerably across the included studies and is likely to reflect varying disease severity and different methods and sites used to measure changes in skeletal muscle. Therefore, it was deemed inappropriate to undertake a meta-analysis. These findings, however, highlight that muscle wasting is not consistent across muscle groups during the acute phase of critical illness and the difficulty with comparing findings between heterogeneous studies.

Delivery of energy above and below an individual's needs may be detrimental to muscle mass. Specifically, caloric restriction can lead to protein-energy malnutrition and muscle wasting, while overfeeding energy may decrease capillary blood flow and amino acid transfer to muscle tissue via perivascular adipose tissue.[41,42] The majority of participants in this review received appropriate energy provision for the acute stages of critical illness compared with measured needs or clinical guidelines.[28] Despite this, the amount of energy delivered to participants did not appear to be associated with changes in skeletal muscle measures. However, there was a large amount of missing data from non-nutrition sources of energy in the included studies, and along with limitations in study design, which are discussed further below, this review has limited capacity to assess the association between energy delivery and skeletal muscle mass changes in acute critical illness.

Protein is crucial for maintenance of muscle in healthy individuals and disease states,[5] and it is likely that appropriate delivery of both energy and protein is important for the attenuation of muscle wasting in critical illness. The optimal level of protein delivery to ICU patients to elicit positive patient outcomes is still to be determined.[43] Recent clinical guidelines suggest that critically ill patients are likely to benefit from a protein intake of at least 1.2 g/kg per day in the context of appropriate energy provision.[29] The evidence base for these recommendations is, however, limited to small observational studies. Only 2 studies in this review provided both energy and protein at levels consistent with current clinical guidelines.[35,36] Even providing 100% of measured energy requirements as well as protein within current recommendations, Uehara et al[36] observed large

losses of TBP in a group of very sick patients, of which half were admitted with sepsis. As reported and discussed by Weijs,[44] even in the context of appropriate energy provision, targeted protein in patients with sepsis may not elicit positive outcomes and may be harmful. In their randomized control trial, Ferrie et al reported small improvements in a number of measures (handgrip strength, fatigue, and muscle thickness) in participants with increased protein delivery; however, this study was not adequately powered to detect a difference in skeletal muscle changes.[35] Puthucheary et al[8] was the only group to report that protein intake was inversely associated with muscle loss. Participants in that study received levels of protein and energy below practice recommendations, and as reported elsewhere,[45] the authors did not adjust for exposure to nutrition delivered which limits the validity of this finding. Additionally, half of the participants in this study were admitted with sepsis, which as discussed above, may have influenced these findings. Overall, based on the results presented in this review, it is not clear how protein delivery impacts skeletal muscle mass changes in the acute stages of critical illness, and further well-designed studies are required. Patients admitted with sepsis should be investigated separately in future studies.

Poorer clinical outcomes have been observed in patients with lower than normal muscularity and those admitted to the ICU with a body mass index <25 kg/m^2 or >35 kg/m^2.[18,19,46] Nutrition support in these high-nutrition-risk groups may lead to more favorable outcomes by maintaining lean body mass, correcting micronutrient antioxidant deficits, and maximizing protein synthesis.[47] The impact of nutrient delivery on muscle mass changes in high-nutrition-risk patient groups was not well described in the studies included in this review, and therefore it was not able to be explored further. An interesting finding included in this review was from Reid et al[12] who compared the participants with the thickest muscles to those with the thinnest muscles, finding that those with the greatest amount of muscle at study commencement lost significantly more muscle thickness during the study period. This may indicate muscle sparing with limited muscularity, but this could also imply a greater possibility of more muscle atrophy in those with high levels of muscularity, and requires further investigation.

Strengths and Limitations of this Systematic Literature Review

This is the first systematic review to investigate the association of energy and protein on direct measures of skeletal muscle mass in critically ill adults. A strength of the review is that data were extracted and synthesized to compare nutrition provision in the included studies against current clinical recommendations. There are limitations with the findings of this review. First, included studies were mostly observational, with small sample sizes and high risk of confounding, specifically not accounting for medications administered, sepsis, organ failure, and immobility. Second, the majority of studies were not adequately powered and were likely too small to detect clinically worthwhile differences. Third, although strength of this review was comparison of nutrition provision by weight, obtaining an accurate weight is difficult in critically ill patients, which may impact the reliability of the findings. Finally, critically ill patients experience large fluid shifts.[30,32,34] The methods used to measure muscularity in this review may therefore be limited by variable tissue hydration, which may affect the accuracy of skeletal muscle mass measures that are presented in this review. A discussion on body composition methods and challenges in the ICU setting was beyond the scope of this review; readers should refer to published reviews for further information.[25,48]

Conclusion

Currently, there is limited evidence to understand the association between energy and protein delivery on skeletal muscle mass changes in critically ill patients. Future studies in this area should be adequately powered, account for all potential confounding factors to changes in skeletal muscle mass, and detail all sources and quantities of energy and protein delivered to participants.

Statement of Authorship

K. J. Lambell contributed to the concept and design of the research; K J. Lambell, A. K. Forsyth, and S. J. King contributed to the acquisition and analysis of the data; K. J. Lambell, A. K. Forsyth, S. J. King, and A. C. Tierney contributed to interpretation of the data; and K. J. Lambell drafted the manuscript. All authors critically revised the manuscript, agree to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final manuscript.

Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Supplementary data (table associated with the manuscript)

Table 5.1 Subject headings and keywords used in database search

Concept A (Population) Critically Ill patients AND	***MESH headings (Ovid)*** • Intensive care OR • Critical Care • Critical Illness • expel multiple organ failure/ or exp shock, cardiogenic/ or exp shock, haemorrhagic/ or exp shock, surgical/ or exp shock, traumatic/ or exp systemic inflammatory response syndrome/ or exp shock, septic/ • Intensive care units/ or burn units/ or coronary care units/ or respiratory care units/ • Respiration, artificial/ • Ventilators, Mechanical/ CINAHL (additional subject headings) • Multiple organ dysfunction syndrome • Ventilated patients ***Key Words*** • All of the above.tw. • Systemic inflammatory response*.tw. • Burn unit*.tw. • Coronary care unit*.tw. • Respiratory care unit*.tw. • Artificial respiration.tw. • Mechanical* ventilat*.tw. • Critical* Ill*.tw. • Mechanical* ventilat*.tw. • ICU.tw. • Organ failure.tw.
Concept B (Intervention) Body composition tools AND	***MESH headings (Ovid)*** • Ultrasonography/ OR • Exp diagnostic imaging/ or exp tomography, x-ray computed/ or exp imaging, three-dimensional/ or exp magnetic resonance imaging/ or exp multimodal imaging/ or exp whole body imaging/ • Absorptiometry, Photon/ • Electric Impedance/ • Neutron Activation Analysis/ Separate

	Key words • All of the above.tw. • Ultraso*.tw. • Magnetic resonan* imag*.tw. • Comput* tomography*.tw. • Dual-energy X-ray absorptiometry.tw. • In vivo neutron activation analysis.tw. • Electric* imped?nce*.tw. • Neutron Activation Analysis.tw. • Skinfold thicknes*
Concept C (Outcome) Skeletal muscle mass	***MESH headings (Ovid)*** • Body Composition/ OR • Muscular Atrophy/ • Sarcopenia/ • Muscle, skeletal/ or quadriceps muscle/ or respiratory muscles/ or diaphragm • Muscle, skeletal/us • Quadriceps muscle/us CINAHL (additional subject headings) • Fat free mass ***Keywords*** • All of the above.tw. • Body composition.tw. • Atroph* adj1 musc*.tw. • Sarcopen*.mp. • Skeletal muscle*.tw. • Lean body.mp. (weight or mass) • Fat free mass.mp. • Muscle thickness.tw. • Muscle layer thickness.tw. • Muscle wasting .tw. • Muscle mass.tw. • Quadricep* muscle*.tw. • Rectus femoris muscle*.tw. • Skeletal muscle*.tw.

5.4 Re-run of the systematic literature review

The search strategy detailed in the manuscript was repeated to include any additional articles published between 1st January 2016 – 27th July, 2020. Specifically, criteria for the inclusion of studies were critically ill adult participants aged 18 years or older admitted to an ICU, where skeletal muscle mass and/or total body protein, were measured on at least two occasions during acute critical illness (up to two weeks after ICU admission). Documentation of mean energy, with or without protein, delivered to participants in the time period between the measurement of skeletal muscle mass and/or total body protein was also required for eligibility. In the original search no study was excluded due to measurements being performed two weeks after ICU admission. For the re-run of the search, we were interested in changes over the entire ICU admission so we did not exclude studies if muscle measurements were performed after two weeks. Quality assessment of included articles was performed by candidate KL and Dr Susannah King using the American Dietetic Association quality checklist (the same as the original review)[147].

5.4.1 Results

The search yielded 379 publications, of which 126 were duplicates, leaving 253 for title and abstract screening against inclusion and exclusion criteria. There were 244 articles excluded at this point, leaving 9 publications for full text review. Of these, five did not report nutrition delivery between muscle assessments, leaving four studies which met all the inclusion and none of the exclusion criteria[90,126,148,149]. Table 5.2 outlines the characteristics of the included studies.

Table 5.2 Characteristics of included studies in systematic review re-run

Author, year, country	No. of participants	Participant demographics[a]	Intervention, study design and aim(s)	Mean energy delivered (kcal/kg/day)[b]	Mean protein delivered (g/kg/d)[b]	Outcome measure and time between measurements	Results - % muscle loss over study period	Comments
McNelly et al, 2020[148] UK	n=127 Intermittent: n=62 Continuous: n=59	Heterogeneous group *Intermittent feeding:* Age 55[51-59], Male 41%, BMI n/a, APACHE II 23[20-26] *Continuous feeding:* Age 60[56-64], Male 40%, BMI n/a, APACHE II 20[18-22]	Intermittent Vs. Continuous EN: **Prospective RCT** to investigate whether delivering intermittent enteral feed would decrease muscle wasting compared with continuous feed in critically ill patients	*Intermittent:* 19.0[17.5-20.4] and *continuous:* 16.8[15.1-18.5]	*Intermittent:* 0.90[0.84-0.96] and *continuous:* 0.86[0.77-0.94]	**Ultrasound:** rectus femoris CSA (cm^2) Measurements: Study day 1, 7, and 10 and at ICU and hospital discharge	Rectus femoris CSA= substantial loss in both groups ~20% at day 10. No difference in percentage loss between intermittent and continuous groups (p=0.505) despite intermittent group meeting significantly higher energy and protein targets	Large amount of missing data at day 10 (31 patients included for intermittent and 32 for continuous) Energy expenditure not measured
Berger et al, 2019[149] Switzerland	n=23 EN + SPN: n=11 EN: n=12	Heterogeneous group *EN + SPN:* Age 63[55-73], Male 82%, BMI 28[26-31], APACHE II 28[26-31] *EN*: Age 67[62-75], Male 83%, BMI 25[24-30], APACHE II 23[19-28]	EN + SPN Vs. EN: **Prospective RCT** to investigate the potential mechanisms underlying the reduction of infectious complications observed in a previous SPN trial	*EN + SPN:* 24.3 and *EN:* 16.1	*EN + SPN:* 1.16 and *EN:* 0.67	**Ultrasound**: rectus femoris CSA (cm^2) Measurements: day 4, 9-10, and 15	Rectus femoris CSA= at day 15, losses tended to be greater in the EN group versus EN + SPN group (-23% versus -16%, p=0.068)	Energy expenditure measured

Table 5.2 Cont.

Fetterplace et al, 2018[90] Australia	n=60 High protein EN: n=30 Standard EN: n=30	Heterogeneous group *High protein EN:* Age 55±13, Male 77%, BMI 30±7, APACHE II 22±6 *Standard EN:* Age 57±16, Male 70%, BMI 29±5, APACHE II 20±6	High protein EN Vs standard care: **Prospective RCT** to 1) investigate whether a high protein volume-based EN protocol delivered more protein & energy than standard EN, & 2) evaluate whether the intervention attenuated muscle or weight loss	*High protein EN:* 23±6 and *Standard EN:* 21±3	*High protein EN:* 1.2±0.3 and *Standard EN:* 0.7±0.1	**Ultrasound:** quadriceps muscle layer thickness (cm) Measurements: ICU day 1-2 and ICU discharge or day 15 (whichever came first)	Quadriceps muscle layer thickness= high protein EN group had less muscle loss at discharge, with a mean attenuated loss of 0.22cm (95% CI, 0.06-0.38; p=0.01)	~25% missing ultrasound data at ICU discharge or day 15 Energy expenditure not measured
Fetterplace et al, 2019[126] Australia	n=60	Heterogeneous group Age 58±16, Male 55%, BMI 28 [28-31], APACHE II 23±8	**Observational study** to 1) determine the cumulative energy deficit from EN/PN using calculated predictive equations and measured energy expenditure, & 2) explore associations between energy deficit & nutritional outcomes (weight and FFM) & functional outcomes at ICU discharge	16±6	0.58±0.25	**Bioimpedance spectroscopy:** FFM (kg) Measurements: Study day 1 and ICU discharge	FFM = mean loss -7.7kg (95% CI -10.0 to -5.0). Greater losses with larger energy deficits (for 1000kcal cumulative deficit subjects lost on average 1.3kg (95% CI=0.2-2.4kg; p=0.02)	Energy expenditure measured for 72% participants, but predictive equations were used to determine energy deficit and relationship with FFM changes

APACHE, acute physiology and chronic health evaluation score; BMI, body mass index; CSA, cross-sectional area; EN, enteral nutrition; FFM, fat-free mass; ICU, intensive care unit; SPN, supplementary parenteral nutrition. [a]data rounded to nearest whole number. [b]Weight reported as mean participant weight (actual or obesity adjusted) or measured weight on day 10. Data presented as median [interquartile range] or mean±standard deviation.

Quality assessment of the included studies was performed using the American Dietetic Association quality checklist [147]. Using this tool, a study will receive a positive rating if the following validity questions are answered "yes" as well as at least one additional "yes":

- Was the selection of study subjects/patients free from bias?
- Were the study groups comparable?
- Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described?
- Were outcomes clearly defined and the measurements valid and reliable?

Three of the studies were randomised controlled trials (RCT) and were all assessed as meeting criteria for a positive rating[90,148,149]. Each used different nutritional strategies aimed at attenuating skeletal muscle wasting, including:

- Intermittent (bolus) versus continuous enteral nutrition (EN) delivery[148];
- EN plus supplemental parenteral nutrition (PN) versus EN alone[149]; and
- A high protein volume based EN protocol versus standard care[90].

The hypothesis for the intermittent versus continuous EN study by McNelly et al, was that the delivery of nutrition as a bolus would result in peaks in amino acid concentrations (rather than low dose continuous concentration) which may in turn stimulate muscle protein synthesis and reduce muscle losses[148]. The other two nutritional strategies were aimed at increasing total energy and protein delivery compared to standard nutritional care[90,149].

The fourth study, which was observational, used bioimpedance spectroscopy (BIS) to observe changes in muscularity in relation to energy delivery[126]. It was assessed as meeting criteria for a neutral rating, as it showed weaknesses in design and interpretation

of data, specifically by not accounting for fluid shifts and the potential impact on FFM values[126]. No studies received a negative rating.

The included studies had modest sample sizes ranging from n=23 to 127. Only one study was powered to detect changes in muscularity[148]. All study populations were heterogeneous, including patients with a range of reasons for ICU admission, which is reflective of usual ICU populations. Most patients had high clinical acuity with a mean Acute Physiology and Chronic Health Evaluation (APACHE II) score at ICU admission of around 20-23 (a score of 25 represents a predicted mortality of 50%).

In the original review, there was a range of body composition methods and anatomical sites used to describe changes in skeletal muscle mass or total body protein across the acute stage of illness (e.g. *in vivo* neutron activation analysis, computed tomography image analysis, and ultrasound). In this update of the review, three of the four studies used ultrasound as the method to longitudinally investigate the impact of nutrition delivery on changes in muscularity[90,148,149]. Two studies measured rectus femoris CSA(cm^2)[148,149], and the other measured quadricep muscle thickness (cm), using maximum compression[90]. The remaining observational study used BIS to observe changes in estimates of FFM[126].

All studies measured changes in muscle indices over the first few weeks of critical illness. Loss of quadriceps musculature ranged from 16% to 23% over the study period. For two of the ultrasound studies, there was a large amount of data missing for follow-up muscle measurements at day 10 and at ICU discharge (approximately 25%)[90,148]. Some of the reasons given for missed observations were: patient not available to perform

measurements, palliation, patients discharged from ICU before the primary investigator could perform measurements, and death[90,148].

In the study using bioimpedance technology, the authors reported a mean difference in body weight from ICU admission to discharge of 3kg (95% CI -5.2 to -0.7) and FFM of 7.7kg (95% CI -10 to -5)[126]. This is an unusual finding which is considered in more detail in the discussion section below.

Energy and protein delivery and impact on muscle mass changes

Participants were delivered nutrition via enteral and/or parenteral nutrition in all studies. Nutrition delivered via the oral route was not recorded, so it is unknown if it was provided or not. Three of the four studies accounted for all sources of energy (IV glucose and propofol)[90,148,149]. Energy targets were based on estimated requirements for two studies[90,148], a combination of estimated and measured requirements (by indirect calorimetry) for one study[126], and solely on measured requirements by indirect calorimetry in the other study[149].

As outlined in Table 5.2, the mean daily energy and protein delivery varied across studies (16 - 24 kcal/kg/day, 0.6 – 1.2 g/kg/day). Of the RCTs, Berger et al, reported lower loss of quadriceps muscle in the intervention group who received EN plus supplemental PN group (24kcal/kg and 1.16g/protein/kg) compared to the EN only group who received less nutrition (16kcal/kg and 0.67g/protein/kg)[149]. However, the sample size was very small (underpowered) and the changes did not reach statistical significance (-16% versus -23%, respectively p=0.068)[149]. In the pilot RCT by Fetterplace et al, lower loss of quadriceps muscle was observed in the intervention who received more nutrition via an enteral high

protein volume-based feeding protocol protocol (23kcal/kg and 1.2g/protein/kg) compared to patients in the standard care group (21kcal/kg and 0.75g/protein/kg) (mean attenuated loss of 0.22cm, 95%CI, 0.06-0.38; p=0.01)[90]. Conversely, in the largest RCT (n=123), McNelly et al reported no difference in percentage quadriceps muscle loss between the groups (intermittent versus continuous enteral nutrition), despite the intermittent group receiving more nutrition and meeting significantly higher energy and protein requirements (*energy*: 82% [95% CI 79-86%] versus 73% [95% CI 69-76%]; p<0.001 and *protein*: 80% [95% CI 77-83%] versus 70% [95% CI 67-73%]; p<0.001)[148]. Whilst the separation in nutrition delivery was statistically significant, it is fairly modest and may not have been sufficient to observe a detectable difference in rectus femoris CSA.

5.4.2 Discussion

The purpose of re-running the original literature search was to provide an update on studies in this area. There were four studies identified. Three were RCTs; all with relatively small sample sizes, and all three used ultrasound to longitudinally measure changes in quadriceps musculature in response to different nutrition strategies. Like previous studies, significant and rapid loss of muscle was observed over the first few weeks of critical illness. As there were different techniques and muscle indices used to assess changes in muscularity across the four studies (and previous studies) it was not appropriate to undertake a meta-analysis.

Interestingly, there was a similar number of studies published in the last four years compared to the entire period in the original search from January 1946 to March 2016. This may be partly explained by the increased recognition of the importance of

maintaining muscle for recovery from critical illness and the focus away from hard clinical outcomes (i.e. such as mortality) and toward more patient-focused functional and quality of life outcomes for critical care nutrition trials[150] . This may have increased the number of studies investigating strategies (such as nutrition delivery) aimed at attenuating muscle losses. It may also be attributable due to the emerging use of ultrasound to easily and rapidly measure changes in muscularity at the bedside at pre-determined time points (compared to techniques employed in the earlier studies which required specialised machinery and expertise for analysis and available at only a few centres).

The three RCTs included in this update used different nutrition intervention strategies aiming to attenuate skeletal muscle wasting in critical illness. Despite using different strategies, the duration of the nutrition intervention delivered in all studies was relatively short (between 4-10 days). It is possible that the separation of nutrition delivery between the control and intervention groups may not have been adequate to detect a difference in change in muscle mass indices. Furthermore, there was a large number of missing ultrasound muscle data in the final analyses. For example, the McNelly et al study was powered on rectus femoris cross-sectional change at day 10[148]. The authors logically attempted to recruit patients at risk of 'persistent critical illness' as these patients experience significant muscle wasting and are at greatest risk of long-term functional disability, and may have received a significant amount of the intervention strategy[54,55]. Despite recruiting 127 patients, the number of patients with muscle data at day 10 was reduced to half the number of patients enrolled in the trial (n=63) and the mean intervention period was 4 days out of the 10 when the patient was fed enterally[148]. To improve participation retention in a study and to achieve longer periods of nutrition delivery, future studies should consider a whole-hospital trial (from ICU admission to

hospital discharge), with a nutrition intervention combining all routes of feeding (enteral, parenteral, and oral nutrition) and measuring muscle indices at pre-determined time-points in ICU and on the ward (e.g. ICU and hospital discharge). An example of such a study is the INTENT-Muscle study (ClinicalTrials.gov NCT04896515). This study is an observational longitudinal sub-study nested within the currently recruiting RCT "Intensive Nutrition Therapy Compared to Usual Care in Critically Ill Adults (INTENT)" (NCT03292237). INTENT is the first multi-centre trial to compare an intensive, individualised nutrition intervention to standard care for the duration of hospital admission in critically ill patients. The aim of INTENT-Muscle is to compare longitudinal changes in muscle health (assessed by bioimpedance and muscle ultrasound) across the whole hospital admission in patients randomised to each arm of INTENT.

The observational study included in this updated systematic review used BIS to observe changes in FFM from ICU admission to ICU discharge[126]. The authors reported a mean loss of body weight of 3kg (95% CI -5.2 to -0.7), and FFM of 7.7kg (95% CI -10 to -5) over the 7-day average ICU length of stay[126]. These findings are surprising, as it is not physiologically possible to store 4kg of fat mass that quickly[66]. Furthermore, the average weight was 82kg (standard deviation, SD 22kg), and FFM 69kg (SD 19). This level of lean tissue according to the body weight (i.e., 16% fat) seems unlikely in a sick population with a median BMI 28 kg/m^2 (IQR 24-31) (i.e. not elite sports players from strength-based sports in whom a phenotype of a relatively high BMI with high fat-free mass is more realistic). As outlined in chapter 1, fluid overload (often seen in early critical illness) can overestimate estimates of FFM and underestimate fat mass (which is calculated as the difference between total weight and fat-free mass). This may be what led to the unrealistic body composition values reported in this paper, and they should be interpreted with caution. This highlights the

importance of carefully reviewing body composition variables derived from bioimpedance technology which may be influenced by multiple factors including fluid shifts, and patient positioning (as discussed in section 1.4.1.2.2). As performed in the original research study presented in Chapter 4, when bioimpedance spectroscopy is used, this is possible by accepting values which are likely appropriate for muscle assessment (e.g. Cole plot follows a half semi-circular pattern, standard error of estimation for fit below 1.0, intracellular resistivity greater than extracellular resistivity, and whole body fat-free mass values are within physiological limits)[119]. Additionally, this also emphasises the need to investigate methods to adjust FFM values for overhydration in acute settings (e.g. Chamney model), also presented in Chapter 4.

5.5 Conclusion

This chapter investigated the association of energy and protein delivery on changes in skeletal muscle mass during critical illness. Overall, despite a growing body of evidence, there is no clearly detectable association between nutrition delivery and skeletal muscle mass changes in critical illness. However, to date studies have been underpowered to detect significant changes in skeletal muscle loss and the nutrition interventions have been delivered in the acute phases of critical illness and for only a relatively short period of time. This reflects the challenges with studying the role of nutrition and muscle outcomes while patients remain in the ICU. Future studies in this area should consider nutrition interventions that are likely to be delivered over longer periods of time and including a primary outcome that is feasible to obtain (i.e. whole-hospital nutrition intervention and allowing collection of muscle measurements if the patients are transferred to the ward). It is also important that these studies aim to collect non-

nutritional variables likely to influence changes in skeletal muscle mass (e.g. injury severity, requirement for organ support, mobility, and medications such as sedation).

The following chapter investigates changes in CT-measured muscle mass and quality during different weeks of critical illness, and the association of clinical and nutrition variables on these changes. The protocol for the retrospective observational study outlined in Chapter 6 was informed by the results and limitations outlined in the original systematic review presented in Chapter 5.

Chapter 6

What changes in skeletal muscle mass and quality occur across different weeks of critical illness and is there an association with energy and protein delivery?

A RETROSPECTIVE OBSERVATIONAL STUDY

6.1 Declaration of authorship

Student's declaration:

The nature and extent of contributions to Chapter 6 of this thesis are as follows:

Name	Nature of contribution	Contribution
Kate Lambell	Study concept and design, ethics application, data collection and analysis, data interpretation, and manuscript preparation and revision for publication	80%
Gerard Goh	Study design, data collection and analysis and revision of manuscript	5%
Audrey Tierney	Study design, data analysis and interpretation, and revision of manuscript	5%
Adrienne Forsyth	Study design, data analysis and interpretation, and revision of manuscript	5%
Susannah King	Study design, data analysis and interpretation, and revision of manuscript	5%

Supervisor's declaration:

I hereby certify that the declaration above is a correct reflection of the extent and nature of contributions made toward Chapter 6 of this thesis by the student and all listed co-authors.

Name of supervisor	**Signature**
Susannah King	

6.2 Introduction

Loss of muscle mass and muscle quality occurs rapidly over the first two weeks of critical illness[11-13]. Changes in muscularity in patients who stay in the ICU beyond this time frame are largely unknown. The causes and mechanisms for muscle wasting in critical illness are multifactorial and may include nutritional inadequacy.

This chapter describes a retrospective observational study which aimed to explore changes in CT-measured skeletal muscle mass and quality (density) across different weeks of critical illness and to investigate associations between changes in these parameters and energy and protein delivery. It also investigates the precision of CT image analysis to detect changes in skeletal muscle mass and quality in the ICU setting. This study was a stand-alone study designed and carried out specifically for the candidate's doctoral research. The work in this chapter relates to thesis objectives 5, 6, and 7:

- To investigate the association between energy and protein delivery and skeletal muscle changes in critical illness.
- To explore the precision of CT image analysis to detect changes in muscle mass and quality in critical illness.
- To explore changes in CT-measured muscle mass and quality according to week of critical illness.

The methods employed in this study are outlined in detail in chapter 2. The results for this chapter are presented in the form of a manuscript accepted and published in *Nutrition (Impact Factor 4.008).* The citation is as follows:

> Lambell KJ, Goh GS, Tierney AC, Forsyth A, Nanjayya V, Nyulasi I, King SJ. Marked losses of computed tomography–derived skeletal muscle area and density over the first month of a critical illness are not associated with energy and protein delivery. *Nutrition*. 2021. DOI: 10.1016/j.nut.2020.111061

Supplementary data (associated with the publication) are presented after the manuscript. Specifically, scatterplots are presented to provide a visual representation of the relationship between changes in CT-measured skeletal muscle CSA and quality and energy and protein delivery and adequacy. A table is also displayed which reports the correlations between changes in skeletal muscle area and quality and clinical and nutrition variables.

6.3 Manuscript

"Marked losses of computed tomography-derived skeletal muscle area and density over the first month of a critical illness are not associated with energy and protein delivery"

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Applied nutritional investigation

Marked losses of computed tomography–derived skeletal muscle area and density over the first month of a critical illness are not associated with energy and protein delivery

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ABSTRACT

Objectives: Changes in muscularity during different phases of critical illness are not well described. This retrospective study aimed to describe changes in computed tomography (CT)–derived skeletal muscle area (SMA) and density (SMD) across different weeks of critical illness and investigate associations between changes in these parameters and energy and protein delivery.
Methods: Thirty-two adults admitted to the intensive care unit (ICU) who had ≥2 CT scans at the third lumbar area performed ≥7 d apart were included in the study. CT-derived SMA (cm^2) and SMD (Hounsfield units) were determined using specialized software. A range of clinical and nutrition variables were collected for each day between comparator scans. Associations were assessed by Pearson or Spearman correlations.
Results: There was a significant decrease in SMA between the two comparator scans where the first CT scan was performed in ICU wk 1 (n = 20; $P < .001$), wk 2 (n = 11; $P < .007$), and wk 3 to 4 (n = 7; P = .012). There was no significant change in SMA beyond ICU wk 5 to 7 (P = .943). A significant decline in SMD was observed across the first 3 wk of ICU admission ($P < .001$). Overall, patients received a mean 24 ± 6 kcal energy/kg and 1.1 ± 0.4 g protein/kg per study day and 83% of energy and protein requirements according to dietitian estimates. No association between SMA or SMD changes and nutrition delivery were found.
Conclusions: Critically ill patients experience marked losses of SMA over the first month of critical illness, attenuated after wk 5 to 7. Energy and protein delivery were not associated with degree of muscle loss.

Introduction

Skeletal muscle is the most abundant tissue in the human body [1]. In addition to its functional role in movement, skeletal muscle has important immunologic and metabolic functions, which play an essential role in the body's ability to respond to illness [1]. Skeletal muscle mass may be an important predictor of outcome in critically ill patients with low muscularity at the time of intensive care unit (ICU) admission being associated with ICU and hospital length of stay (LOS) and mortality [2,3]. In addition to muscle mass, a decline in muscle quality (characterized by adipose or fibrous tissue infiltration into the muscle) may be a critical factor in the ability for an individual to recover from critical illness [4,5].

Muscle wasting is of significant concern in critical illness with rapid and significant muscle losses reported over the first 2 wks in intensive care, resulting from muscle protein breakdown exceeding muscle protein synthesis [6,7]. The level of muscle depletion in patients who stay in the ICU beyond this time frame is mostly unknown. The causes and mechanisms for muscle wasting in critical illness are likely multifactorial and still being investigated, but may include altered substrate metabolism, anabolic resistance, hypoxia, inflammation, immobilization, and nutritional inadequacy [7–11].

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Despite the importance of muscle mass and muscle quality in recovery from critical illness, the noninvasive assessment of these indices is challenging and many traditional body composition methods are not feasible in ICU settings (e.g., dual-energy x-ray absorptiometry) [12]. In recent years, computed tomography (CT) scans performed for clinical purposes have been utilized to assess muscle health [13]. Specifically, quantification of skeletal muscle area (SMA) at the third lumbar (L3) region is highly related to whole-body muscularity and considered a reliable and precise method for muscle mass assessment in non-ICU (predominantly oncologic) populations [12]. Furthermore, CT image analysis allows for the assessment of skeletal muscle density (SMD), a marker of muscle quality [5].

The aims of this study were to (1) describe changes in SMA and SMD determined by the analysis of CT images performed ≥7 d apart in critically ill patients across different weeks of ICU admission, and (2) investigate associations between changes in SMA and SMD and energy and protein delivery and other clinical variables.

Methods

Study design and setting

This retrospective, observational, single-center study used information from electronic medical records for patients admitted between February 2009 and July 2019 to the ICU at The Alfred Hospital, an adult tertiary teaching hospital in Melbourne, Australia. The research and ethics committees at The Alfred and La Trobe University approved the study protocol, and a waiver of consent was approved.

Participants

Adult patients (age ≥18 y) admitted to the ICU who had a CT scan for routine clinical care ≤24 h before or during ICU admission and a second (or multiple) CT scan(s) ≥7 d later were screened for inclusion. The 7 d minimum interval between scans was chosen as suitable to detect changes in SMA and SMD via CT image analysis if changes were to occur. Patients were included if both CT scans were appropriate for analysis of SMA and if the predominant nutrition route was enteral and/or parenteral (planned >70% requirements) due to oral intake not routinely recorded in a quantifiable manner.

Participant and clinical characteristics

Age, sex, Charlson comorbidity index [14], Acute Physiologic and Chronic Health Evaluation (APACHE) II [15] and III [16] score, admission diagnosis (trauma, medical, or surgical), ICU and hospital LOS, and in-hospital mortality were recorded. Weight, height, and body mass index (BMI; kg/m^2) were also recorded using estimated/reported values documented by an ICU dietitian at the time of the initial assessment. For patients with a BMI ≥30kg/m^2 an adjusted body weight (actual body weight − ideal body weight [weight at BMI 25] + 25% excess weight) was used to calculate nutritional delivery.

Computed tomography image analysis

CT scanning was performed per standardized hospital protocols, which were consistent throughout the study period. After extraction of the images, a trained investigator (KJL) identified the CT slice at the L3 level for uploading onto software for analysis (sliceOmatic version 5.0, Tomovision, Montreal, QC, Canada), which enabled the skeletal muscle to be identified using boundaries in Hounsfield units (HU; −29 to +150 HU) [17]. SMA (cm^2) was automatically computed by the software by summing the skeletal muscle tissue pixels and multiplying by the surface area of each pixel. Our group has previously reported acceptable reliability values for this purpose in patients admitted to the ICU [18]. Specifically, the mean coefficient of variation (CV) for intrarater and interrater was 0.7% and 0.8%, respectively.

The software automatically computed SMD by calculating the mean radiologic muscle attenuation of all muscle visible at the L3 level, measured in HU. Because this measurement can be altered by the administration and phase of contrast during CT scanning [19,20], patients were excluded from the SMD analyses if the comparator scans did not have a similar contrast administration (as determined by an experienced radiologist, GSG).

The short-term precision of CT image analysis to assess muscle changes was evaluated by identifying and collecting data for 10 adult ICU patients who had 2 CT scans at the L3 area for clinical purposes, performed ≤24 h apart (i.e., a period where significant changes in SMA and SMD would not be expected). The mean time between the scans was 12 ± 5.3 h (range, 5−21 h). The mean SMA values for CT1 and CT2 were 160.5 ± 53.6 cm^2 and 162.7 ± 50.8 cm^2, respectively (P = .399), and for CV was 3.21% (range, 0.78%−5.34%). Six patients had CT scans with comparable contrast administration, enabling the determination of SMD precision. The mean SMD values for CT1 and CT2 were 40.3 ± 11.5 HU and 42.3 ± 10.3 HU, respectively (P = .025), and for the mean CV was 4.56% (range, 0.83%−10.10%).

Documentation of nutrition delivery and potential confounders

Routinely documented variables thought to potentially impact muscle changes were collected for each day between the comparator CT scans. Mean daily values were calculated to enable a comparison given the variability in the number of days between the CT scans. The variables are detailed in Table 1.

Energy (kcal) and protein (g) requirements were recorded for each day between the CT scans using estimates determined by the ICU dietitian(s) managing the patient's nutrition care (and not involved in the study). Delivered energy and protein were calculated from all possible sources (EN, PN, intravenous dextrose, propofol, and intravenous amino acid supplementation). Volumes of EN discarded due to intolerance (high gastric residual volume) were subtracted from the delivered volume. Daily adequacy (%) was calculated for each of energy and protein as follows: (daily amount delivered)/(daily estimated requirements) × 100. Adequacy over the study period was calculated as the total adequacy divided by the number of study days. Mean energy and protein delivered per kg actual or adjusted body weight (for patients with BMI ≥30 kg/m^2), averaged per study day, were also recorded.

Other potential confounders

Days receiving mechanical ventilation, continuous renal replacement therapy, extracorporeal membrane oxygenation, sedative agents, insulin, or inotrope and/or vasopressor agents were recorded. At the institution's ICU, blood glucose levels are maintained at <10 mmol/L using an insulin titration protocol. Days receiving each therapy were expressed as a percentage of the study days. The ICU mobility scale (0−10) [21] was recorded for each study day using data from the medical record, and averaged across the study period.

Statistical analyses

A sample size calculation was not possible due to the lack of adequately powered studies investigating the association of nutrition delivery on CT-derived skeletal muscle changes in critically ill patients. We included all patients with eligible scans going back 10 y where CT scans were readily available and the scanning methodology was relatively consistent. Shapiro-Wilk tests were used to assess normality. Data are reported as n (%), mean and standard deviation (± SD) or median and interquartile range.

To examine SMA and SMD changes during different phases of critical illness, patients were grouped according to the week that the first CT was performed, and percentage changes in SMA and SMD were calculated and expressed as change per day. A paired samples t test was used to assess differences between the two comparator scans. To investigate the relationship between changes in muscle health and clinical and nutrition variables, the percentage SMA and SMD change per study day, calculated from the first to the last scan, was used. Associations were assessed by Pearson or Spearman correlations. Muscularity status (normal or low) in the first week of critical illness was determined using published cutpoints (<110 cm^2 for women; <170 cm^2 for men) [2].

We also performed a repeated measures analysis in patients who had ≥3 CT scans by fitting a linear mixed model to calculate the mean change in SMA and specifying the number of weeks since admission as the fixed effect and patient as

Table 1
Participant characteristics and clinical and nutrition variables collected

Baseline	Each day between comparator CT scans
Age	Mechanical ventilation (yes/no)
Sex	CRRT (yes/no)
Weight (kg)	ECMO (yes/no)
Height (m)	Sedation (yes/no)
BMI	Insulin (yes/no)
APACHE II and III	Vasopressors and/or inotropes (yes/no)
Charlson comorbidity index	ICU mobility scale (0-10)
Reason for admission	Estimated energy requirement (kcal)
Pre-ICU admission location	Estimated protein requirement (g)
	Delivered energy [EN/PN, dextrose & propofol] (kcal)
	Delivered protein [EN/PN, IV amino acids]

APACHE, Acute Physiological and Chronic Health Evaluation; BMI, body mass index; CRRT, continuous renal replacement therapy; ECMO, extracorporeal membrane oxygenation; EN, enteral nutrition; ICU, intensive care unit; PN, parenteral nutrition; IV, intravenous.

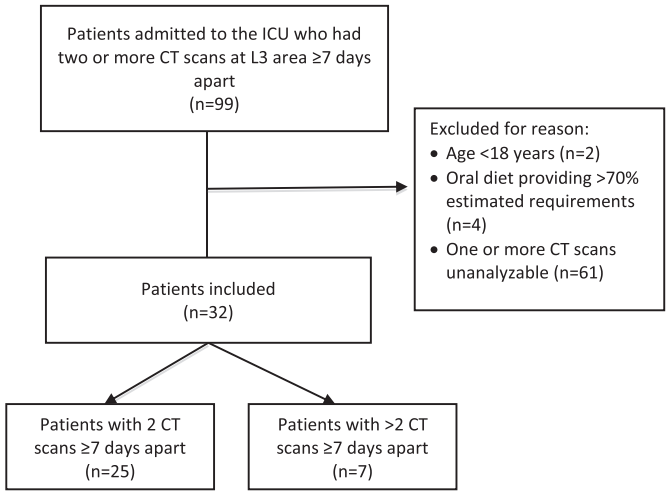

Fig 1. Recruitment flowchart. CT, computed tomography; L3, third lumbar area.

random effect. The statistical analyses were performed using IBM SPSS, version 25 (Armonk, NY) and Stata SE, version 15.0 (StataCorp, College Station, TX). Significance was set as a *P*-value of < .05.

Results

Ninety-nine patients had ≥2 CT scans (at L3 area) performed ≥7 d apart during an ICU admission and were screened for inclusion. Two patients were age <18 y, four patients did not have all nutrition provision recorded, and for 61 patients, ≥1 CT scans did not meet the predetermined quality standards for SMA analysis. Of the 32 patients included, 25 had 2 CT scans and 7 had ≥3 CT scans (Fig. 1).

Patient characteristics are detailed in Table 2. The majority of patients were male (72%), medical admissions (66%), and had high clinical acuity (mean APACHE II score 21 ± 8; median ICU LOS 35 d [range, 22–60 d]).

Changes in skeletal muscle area according to week of critical illness

Table 3 outlines mean SMA across different weeks of ICU admission, and Figure 2 provides a visual representation of these changes. Twenty patients had a CT scan at wk 1 (day 0–7) and a second CT scan ≥7 d later, with a significant decrease in SMA (149.9 ± 38.8 vs. 127.9 ± 38.4 cm^2; $P < .001$). Eleven patients had a CT scan at wk 2 (d 8–14) and a second CT scan ≥7 d later, with a significant loss of SMA (132.8 ± 37.4 vs. 121.8 ± 30.6 cm^2; $P < .007$). Seven patients had a CT scan at wk 3- 4 (d 15–28) and a second CT scan ≥7 d later, with a significant loss of SMA (121.7 ± 35.2 vs. 113.3 ± 35.3 cm^2; $P = .012$). Six patients had a CT scan at wk 5 - 7 (d 36–49) and a second CT scan ≥7 d later, and SMA did not significantly change over this period (98.3 ± 24.5 vs. 98.4 ± 26.7 cm^2; $P = .943$). On repeated measures analysis of 7 patients who had ≥3 CT scans during their ICU stay, the mean reduction in SMA per wk of ICU admission was 6.2 cm^2 (95% confidence interval, 4.39–8.08; $P < .01$).

Table 2
Characteristics of patients admitted to the ICU with ≥2 CT scans ≥7 d apart

Characteristics	All patients (n=32)
Age, y, mean ± SD	54 ± 14
Sex, n (%)	
Male	23 (72)
Female	9 (28)
APACHE II, mean ± SD	21 ± 8
APACHE III, mean ± SD	84 ± 30
Height, m (range)	1.75 (1.70–1.78)
Weight, kg, mean ± SD	87 ± 15
Body mass index, kg/m^2, mean ± SD	29 ± 5
Underweight, n (%)	1 (3)
Normal weight, n (%)	4 (13)
Overweight, n (%)	16 (50)
Obese, n (%)	11 (34)
Charlson comorbidity index, mean ± SD	2 ± 2
Admission reason, n (%)	
Medical	21 (66)
Trauma	6 (19)
Surgical	5 (15)
Pre-ICU admission location, n (%)	
Home	16 (50)
Ward/another hospital	16 (50)
In hospital before ICU, d (range)	5 (2–21)
ICU LOS, d (range)	35 (22–60)
Hospital LOS, d, mean ± SD	68 ± 37
In-hospital mortality, n (%)	9 (28)

APACHE, Acute Physiology and Chronic Health Evaluation; ICU, intensive care unit; LOS, length of stay; CT, computed tomography; SD, standard deviation.

Changes in skeletal muscle density according to week of critical illness

Fifteen patients (75%) who had a CT scan at week 1 and a second scan ≥7 d later met the criteria for assessment of SMD changes, with a significant reduction in SMD (34.6 ± 11.6 vs. 27.7 ± 10.2 HU; $P < .001$; Table 4). Seven patients with a CT scan at week 2 and the second scan ≥7 d later met the criteria for SMD assessment, with no significant change in SMD (29.6 ± 14.7 vs. 27.1 ± 9.6 HU; $P = .311$). At weeks 3 and 5 to 7, there were only 2 and 3 patients, respectively, who had comparable scans for SMD assessment. These data sets were deemed too small for further analyses.

Correlation between changes in SMA and SMD and nutrition and clinical variables

SMA loss was not significant in CT scans performed beyond weeks 5 to 7; thus, data at these timepoints were not included in

Table 3
Changes in SMA according to wk of ICU admission

Wk of first CT scan	n	No. of d between CT scans	First CT SMA (cm^2)	Subsequent CT SMA (cm^2)	Mean difference (cm^2)	*P*-value	% SMA change per study d (cm^2)
Wk 1 (d 0–7)	20	10 (8–15)*	149.9 ± 38.8	127.9 ± 38.4	−21.9 [−29.9 to −13.9]†	< .001	−1.27 ± 0.88
Wk 2 (d 8–14)	11	10 ± 2	132.8 ± 37.4	121.8 ± 30.6	−11.06 [−18.4 to −3.8]†	.007	−0.67 ± 0.77
Wk 3–4 (d 15–28)	7	10 ± 4	121.7 ± 35.2	113.3 ± 35.3	−8.38 [−14.2 to −2.6]†	.012	−0.69 ± 0.66
Wk 5–7 (d 29–49)	6	10 ± 3	98.3 ± 24.5	98.4 ± 26.7	0.16 [−5.7 to 5.4][b]	.943	−0.05 ± 0.81

CT, computed tomography; HU, Hounsfield unit; ICU, intensive care unit; SMA, skeletal muscle area.
Values are reported as mean ± standard deviation unless indicated otherwise.
*Data presented as median (interquartile range).
†Data presented as mean difference [95% CI]. Paired *t* test was used.

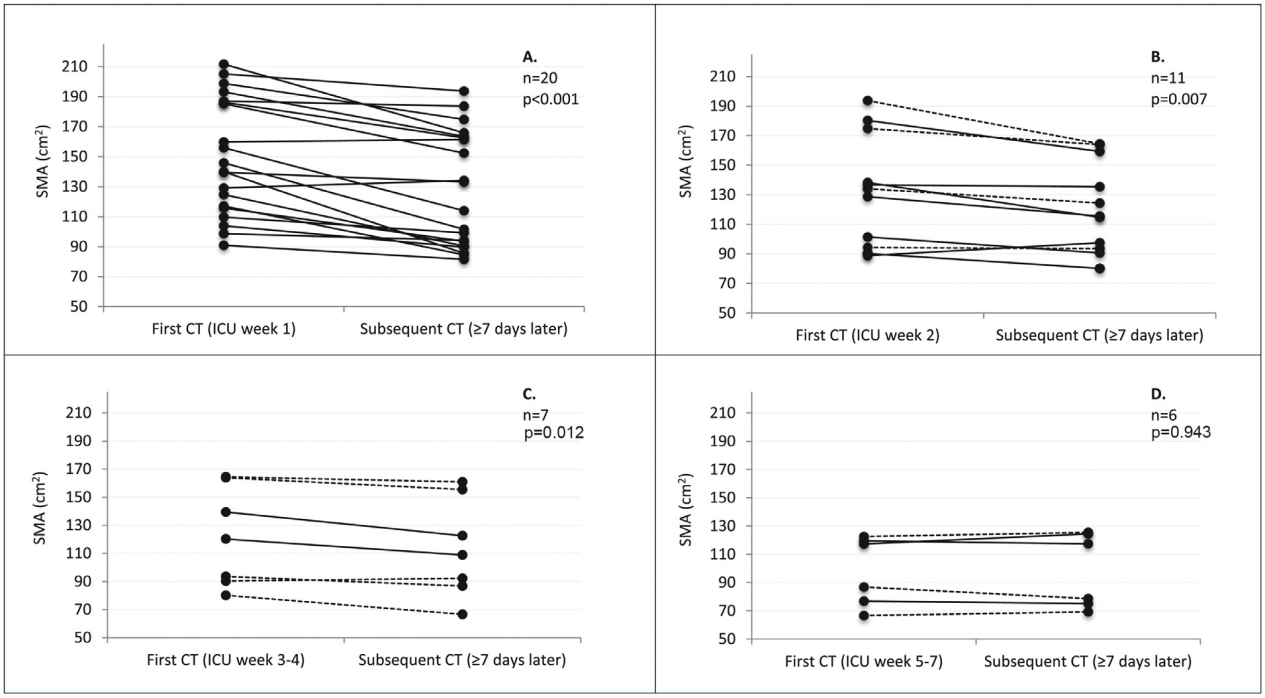

Fig 2. SMA changes according to wk of critical illness from (A) week 1 to week 2 - 3, (B) week 2 to week 3 - 4, (C) week 3 - 4 to week 4 - 6, and (D) week 5 - 7 to week 6 - 9. CT, computed tomography; SMA, skeletal muscle area; SMD, skeletal muscle density. *P*-value is the statistical difference between SMA in the first and subsequent CT scan. Solid line indicates unique patient, dashed line is a patient who had an earlier CT scan included in the analysis (>2 CT scans included).

the correlation analysis. Twenty-eight patients were included, with a median of 13 d (range 9–18 d) between the first and last CT scan (up to week 4) and a mean SMA daily loss of 1.09% ± 0.88%. For correlations with SMD, 19 patients were included, with a median of 11 d (9–17 d) between CT scans, and a mean daily loss of 1.99% ± 2.45% SMD.

The mean energy delivered was 1876 ± 467 kcal/d (24 ± 6 kcal/kg/d), with 83% ± 18% energy adequacy. The mean protein delivered was 89 ± 27 g/d (1.1 ± 0.4 g/kg/d), with 83% ± 23% protein adequacy. Patients received a mix of enteral (88% [range, 53%–94%] study d) and parenteral nutrition (39% [range, 0%–82%] study d). Energy delivery and adequacy were not associated with SMA changes (r = −0.018; P = .928 and r = 0.049; P = .805, respectively). Protein delivery and adequacy were also not associated with SMA changes (r = 0.160 and P = .417 for both). Scatterplots are displayed in Supplementary Figures S1 and S2. Two clinical variables were significantly negatively associated with changes in SMA: Percentage of study days receiving insulin (r = −0.517; P = .005) and sedatives (r = −0.444; P = .018; Fig. S3). SMA changes were not significantly different in patients diagnosed with or without diabetes at the time of ICU admission (mean difference: −0.53 cm^2 [95% confidence interval, −1.28 to 0.20]; P = .151). No nutrition or clinical variable was associated with percentage SMD change per study day. Supplement Table S1 outlines the correlation between all nutrition and clinical variables and percentage change in SMA and SMD per study d. Additionally, when the data were stratified according to the week the first CT was performed, no differences emerged in the correlations between SMA and SMD changes and nutrition variables (data not shown).

Men and women lost muscle at a similar rate (−1.12% ± 0.82%/d vs. −1.03% ± 1.08%/d, respectively; P = .808). Age and BMI at the time of ICU admission were not associated with percentage change in SMA (Suppl. Table S1). Of the 20 patients who had the first CT scan performed <7 d after ICU admission, 11 (55%) had low CT muscle area. These patients had comparable SMA losses to those classified as having normal muscle stores at the time of ICU admission (−1.35% ± 1.05% vs. −1.19% ± 0.57%; P = .686).

Discussion

The assessment and tracking of muscularity during critical illness is imperative to understand the trajectory of change and investigate the effectiveness of interventions aimed at attenuating the marked losses that occur. Our analysis of the short-term precision of CT for SMA and SMD assessment in critical illness suggests that the technique has appropriate short-term precision. We found a considerable reduction of muscle in the first few weeks of critical illness and muscle loss continued to be significant over the first month in the ICU. A

Table 4
Changes in SMD according to wk of ICU admission

Wk of first CT scan	n	No. of d between CT scans	First CT SMD, HU	Subsequent CT SMD, HU	Mean difference, HU	*P*-value	% SMD change per study d, HU
Wk 1 (d 0–7)	15	8 (7–15)*	34.6 ± 11.6	27.7 ± 10.2	−6.81 [−9.45 to −4.17]†	< .001	−2.88 ± 2.51
Wk 2 (d 8–14)	7	10 ± 2	29.6 ± 14.7	27.1 ± 9.6	−2.52 [−8.09 to 3.05]†	.311	−0.41 ± 1.45

CT, computed tomography; HU, Hounsfield Units; ICU, intensive care unit; SMD, skeletal muscle density Values are reported as mean ± standard deviation unless indicated otherwise.
*Data presented as median (interquartile range).
†Data presented as mean difference [95% confidence interval]. Paired *t* test was used.

significant decline in SMD was observed in the first few weeks, but not in the following weeks. A greater number of days of administration of insulin and sedatives were associated with more SMA loss, but energy and protein delivery and adequacy were not.

Several small studies have described changes in muscularity during critical illness using a CT image analysis at the L3 area [22–26]. Two of these studies found no significant wasting over the first 2 wk of critical illness [24,26]. In a study of 33 acute respiratory failure patients, SMA loss was 0.49% per d, although the stage of hospitalization that the CT scans were performed is unclear [25]. Our finding of 1.27% loss of SMA per d is not dissimilar to losses reported in prospective studies measuring quadriceps musculature using ultrasonography (~1.7%/d) [7]. Previously, muscle depletion around the abdomen region was thought to not be as marked as that of the lower limbs [24], so our results shed new light on the patterns of muscle loss. The significant changes observed in the current study may be partly due to high patient acuity because we included patients having prolonged ICU and hospital LOS. Furthermore, we only included data where the CT scans were performed ≥7 d apart, which may explain the greater muscle loss than studies with shorter and/or undefined timepoints between scans. The current study indicates that if CT scans at L3 are performed for clinical reasons (≥7 d apart), they may be a useful indicator of muscle loss and may help guide nutrition management during and beyond the ICU stay. However, as indicated during screening, relatively few patients had two analyzable scans performed at these timepoints, which highlights the importance of studies validating easily applied bedside methods for muscle assessment, such as ultrasound and bioimpedance technology.

There is a paucity of data investigating changes in muscularity in patients staying in ICU longer than 2 wks. One study reported a considerable greater decrease in ultrasound-derived quadriceps muscle thickness over the first 2 to 3 wks compared with later timepoints [27]. In another study, muscle protein turnover between d 10 and 40 of the ICU stay identified that leg muscle protein loss was attenuated between d 30 and 40 compared with the initial phase of the critical illness, which is likely the result of increasing muscle protein synthesis over time [28]. To our knowledge, no studies have reported changes in muscularity across different weeks of critical illness using CT image analysis. In the current study, we observed significant muscle wasting in chronically critically ill patients in the first month, after which SMA loss appeared to be attenuated, which is similar to the muscle protein turnover results mentioned earlier [28]. This highlights that patients who remain in the ICU beyond 2 wks may continue to lose muscle for the first month and, as a result, are at a high risk of malnutrition and long rehabilitation periods. It is critical that patients who stay long-term in the ICU have ongoing evaluations of nutritional status and adequacy to prevent further exacerbation of malnutrition.

Nutrition is delivered to critically ill patients on the premise that it may help attenuate loss of lean tissue during a state of hypermetabolism and catabolism and consequently improve patient outcomes. However, there are only a few small studies investigating the association of energy and protein delivery on muscularity during critical illness (mostly in the first 10 d), with differing findings when reviewed systematically [29]. There are limited data on the impact of nutrition delivery on muscle changes beyond the first 2 wks of critical illness, which may be when the body can metabolically process nutrients [30]. However, research in this area is challenging because there are no available biological marker(s) to determine when an individual shifts from the catabolic to anabolic phase of recovery [31]. We did not find any relationship between energy and protein delivery (and adequacy) and SMA loss. Furthermore, of note, our patients were overall well-fed (24 ± 6 kcal and 1.1 ± 0.4 g protein/kg/d) compared with international registry data (15 ± 8 kcal and 0.6 ± 0.4 g protein/kg/d) [32]. Therefore, we cannot speculate what changes in muscularity would be seen in groups of patients who experience more marked deficits of energy and/or protein delivery, and/or higher protein intake (>1.3 g/kg/d). Moreover, of note, ICU patients on oral diet routinely receive significantly inadequate nutrition [33], and muscle changes in this high nutrition risk group are not captured in the current study. Despite these unknowns, nutrition intervention alone may not be able to attenuate muscle wasting in the context of significant hypoxia, inflammation, and mitochondrial dysfunction in acute critical illness, at least in the first wk of critical illness [10]. Future prospective randomized trials are required to understand the impact of nutrition on recovery when delivered both in the ICU and across the entire hospital stay, as well as in combination with physical activity.

The causes of muscle wasting in critical illness are complex and multifactorial. In this retrospective, observational study using routinely collected variables, we found that the proportions of study days on which sedatives and insulin were administered were related to SMA loss. Not surprisingly, muscle disuse (which occurs in the context of sedative treatment) correlates with greater muscle loss. Stress hyperglycemia and insulin resistance are common in critical illness and thought to involve an adaptive mechanism that prioritizes the utilization of energy to vital tissues [34]. Furthermore, physical inactivity and bed rest are associated with insulin resistance, with decreased insulin action at the muscle level on glucose uptake and glycogen storage [35]. Insulin resistance is also a marker of systemic inflammation and disease severity. Impaired insulin signaling is associated with decreased protein synthesis, which may explain the association with muscle loss we observed [8]. Hyperglycemia and insulin delivery may be important variables to monitor in future prospective studies investigating muscle loss in critically ill patients.

In addition to muscle mass, muscle quality may be important for ICU recovery because of its relation to the functional capacity of skeletal muscle [4]. Although CT scans at L3 can be used to measure and track changes in SMD, radiology expertise is required to ensure that comparator scans are appropriate for assessment. Furthermore, the number of scans appropriate to be quantitatively analyzed for SMD changes is likely to limit the widespread application of this method. A number of studies using ultrasound scans have reported a significant deterioration in muscle quality (echogenicity) at the quadriceps over the first 2 wks of critical illness [4,6]. There are limited studies using CT image analysis to describe changes in SMD in critical illness. Two studies observing changes in the initial phases of a critical illness reported no significant SMD changes [24,26]. Another study of 63 surgical patients in the ICU reported that 34 patients experienced a decrease in SMD of the psoas muscle, whereas the remaining 29 patients had an increase in SMD [22].

In the current study, we reported a significant decline in SMD over the first 2 wk, but no significant changes beyond this period. As with the SMA findings, the reasons for our observation compared with those of other studies may be due to the high severity of illness in our cohort and the defined 7-d period between comparator scans. Whether previous studies excluded patients where the contrast administration in the two scans was not comparable is unclear. There are limited data to interpret the impact of nutrition and other clinical variables on SMD changes. In the current study, we did not find any variable correlated with SMD changes. Future prospective studies should utilize easily applied bedside methods, such as ultrasound

scans, to better understand the influence of nutrition and clinical variables on changes in the muscle quality and how these changes relate to important clinical and functional outcomes [4].

This study has limitations. The retrospective design cannot confirm directionality of associations and precluded the collection of functional parameters, such as muscle strength. Energy expenditure was not routinely measured so the determination of energy adequacy was directed by dietitian estimates. Despite searching medical records for the previous 10 years, the final sample size was modest and patients were sick enough to require multiple CT scans for clinical purposes, which may impact the study's generalizability to all critically ill patients. Furthermore, the sample size prevented statistically powered analyses to understand whether changes in muscularity differed according to the reason for admission. Whether SMA and SMD measured via a single-slice at the L3 are representative of whole-body values also remains unclear. The strengths of the study include its novel findings of the utility of a CT image analysis to measure SMA and SMD changes in critical illness over a longer period of time than previous studies, and the assessment of short-term precision to confirm that longer term changes were unlikely to be explained by poor precision of the technique. Furthermore, using a reference method of body composition analysis (CT image analysis), this study provides valuable insights into the patterns of skeletal muscle changes that occur in chronically critically ill patients.

Conclusions

In this exploratory study, we observed significant muscle loss over the first month of critical illness, with attenuation observed from wk 5 to 7. Nutrition delivery and adequacy were not associated with muscle loss, with patients generally well-fed. These findings highlight the marked changes in skeletal muscle that occur across multiple weeks of critical illness and the critical need for future research to evaluate interventions aimed to attenuate these losses and reverse them during recovery and rehabilitation. This study also emphasizes challenges with performing adequately powered studies using CT scans to measure muscle health and the need for future studies using easily applied bedside methods for muscle assessment to get larger and more heterogeneous study populations.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.nut.2020.111061.

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Figure 6.1 Correlation between *energy* variables and percentage skeletal muscle area change per day in 28 critically ill patients

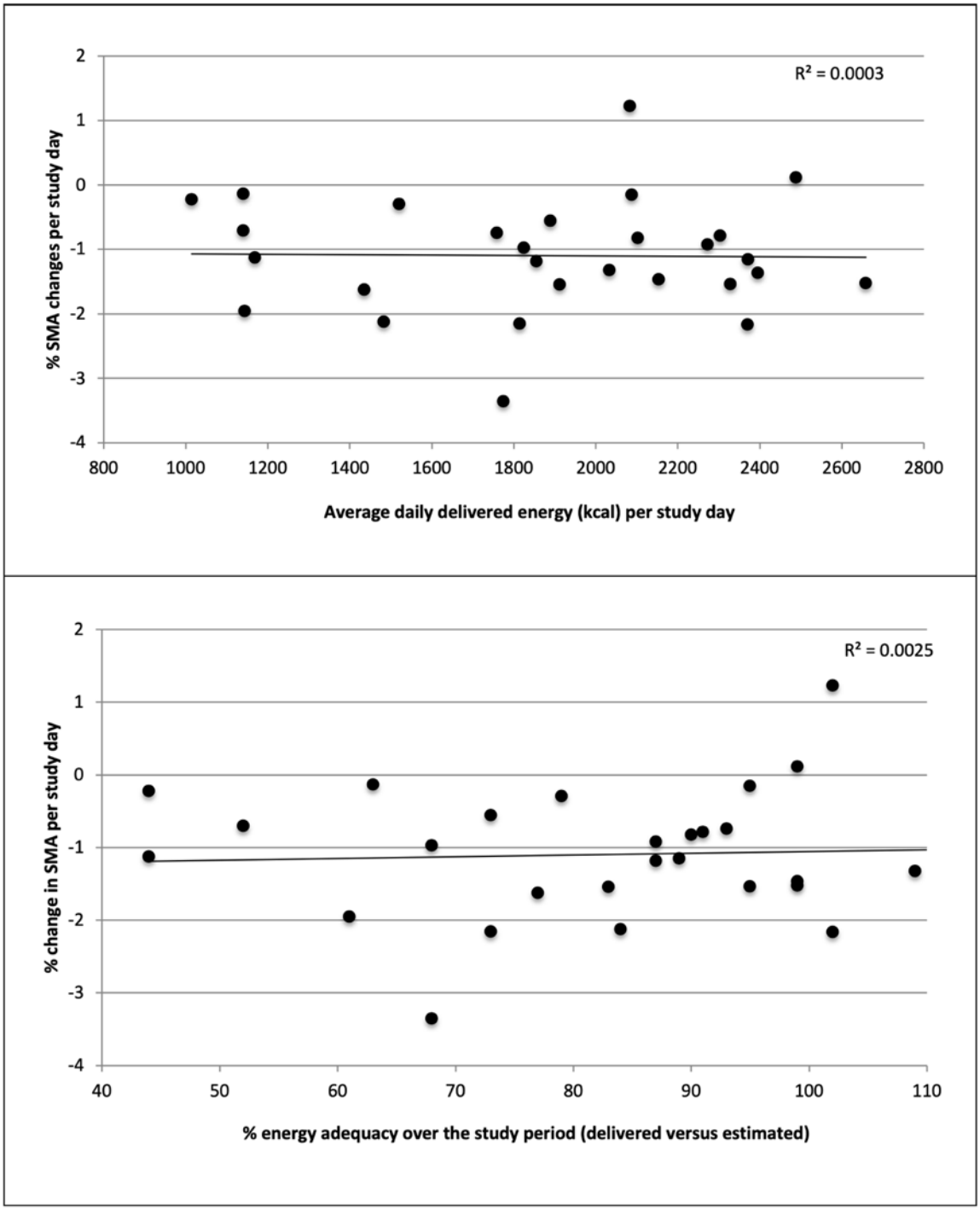

Figure 6.2 Correlation between *protein* variables and percentage skeletal muscle area change per day in 28 critically ill patients

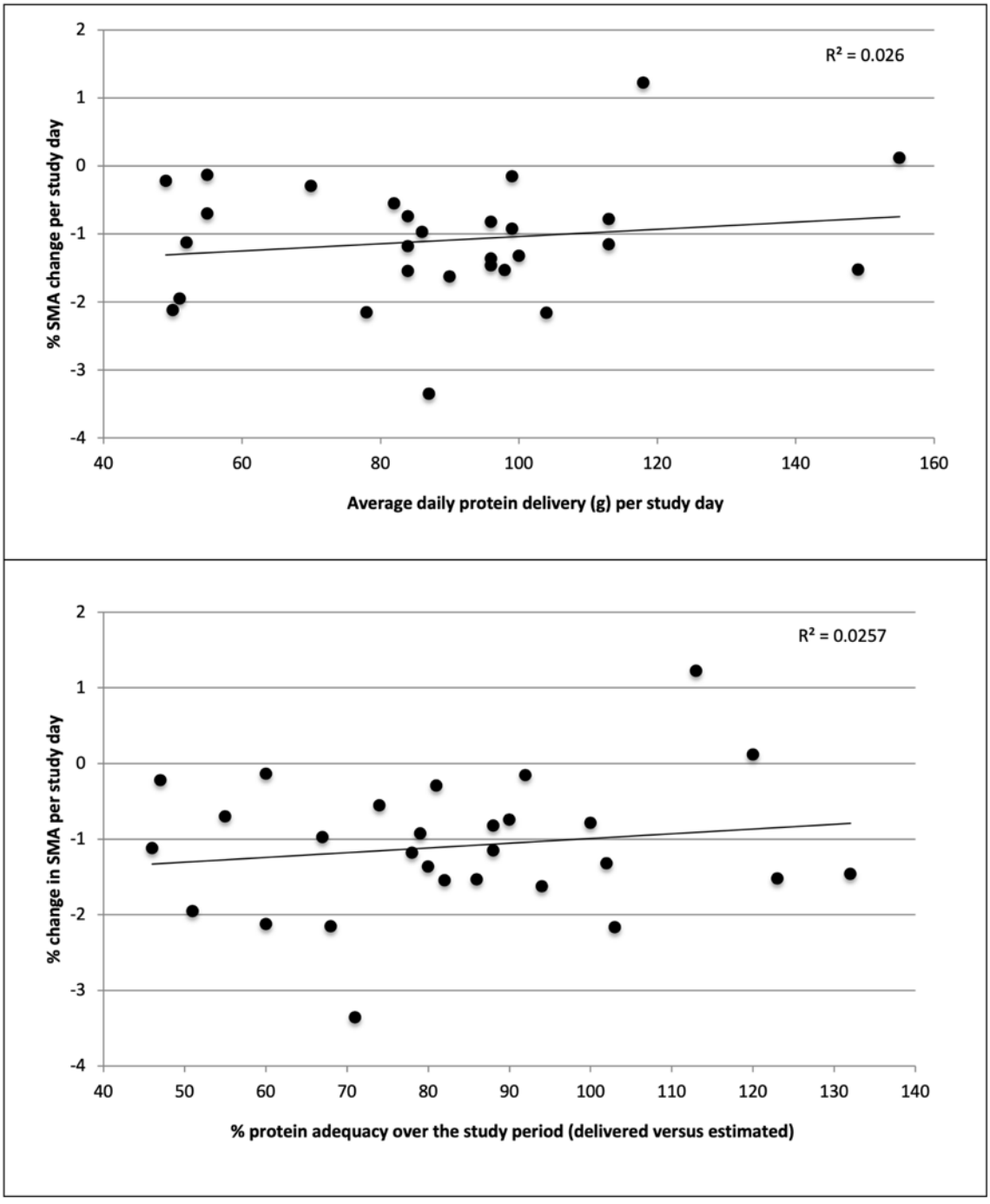

Figure 6.3 Correlation between insulin and sedative administration and skeletal muscle area changes per study day in 28 critically ill patients

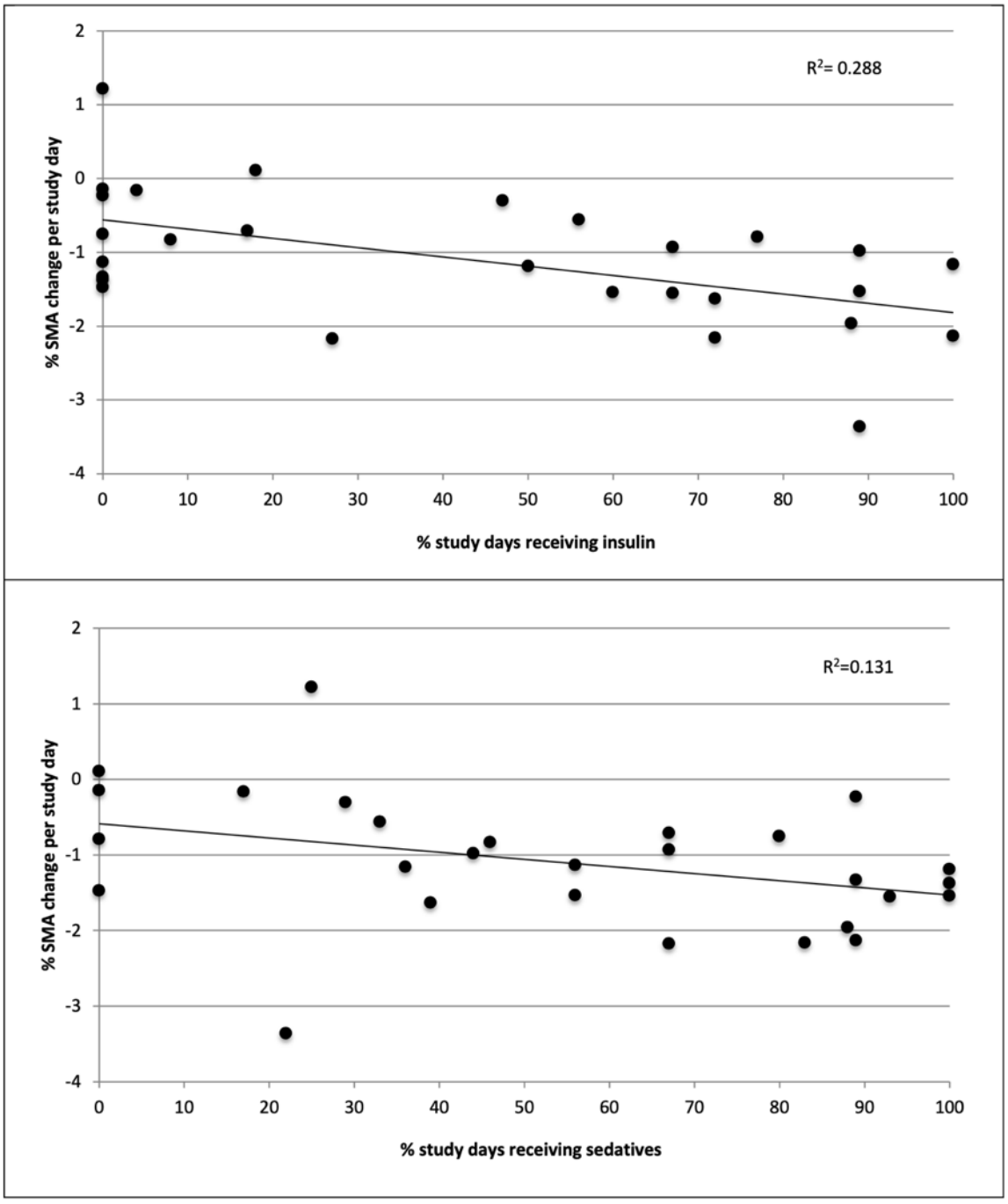

Table 6.1 Correlation between muscle changes and nutrition and clinical variables

		Correlation **% SMA** change per day (n=28)		Correlation **% SMD** change per day (n=19)	
Nutrition variables	**Mean/median**	**Correlation**[b]	**P value**	**Correlation**[b]	**P value**
Energy (kcal) delivered per study day	1876 ± 467	-0.018	0.928	0.241	0.321
Energy (kcal) delivered per/kg/day[a]	24 ± 6	-0.057	0.773	0.192	0.430
Energy adequacy (%)	83 ± 18	0.049	0.805	0.181	0.459
Protein (g) delivered per study day	89 ± 27	0.160	0.417	0.370	0.119
Protein (g) delivered per/kg/day[a]	1.14 ± 0.35	0.124	0.529	0.292	0.226
Protein adequacy (%)	83 ± 23	0.160	0.417	0.396	0.093
% study days receiving EN	88 [53-94]	-0.028	0.889	-0.103	0.678
% study days receiving PN	39 [0-82]	0.068	0.732	0.419	0.074
Clinical variables					
Age, years	54 ± 14	-0.114	0.562	-0.287	0.233
APACHE II	21 ± 8	-0.148	0.451	-0.298	0.215
Charlson Comorbidity Index	2 [0-4]	-0.184	0.347	-0.146	0.551
BMI, kg/m^2	29 ± 5	0.119	0.545	0.180	0.462
% study days receiving MV	94 [70-100]	-0.057	0.774	-0.072	0.769
% study days receiving CRRT	60 [0-91]	-0.087	0.659	-0.258	0.286
% study days receiving ECMO	0 [0-0]	-0.181	0.356	0.049	0.841
% study days receiving insulin	49 [0-75]	-0.517**	0.005	-0.278	0.249
% study days receiving sedatives	56 [27-89]	-0.444*	0.018	-0.355	0.135
% study days receiving vasopressors/inotropes	75 [20-96]	-0.215	0.271	-0.344	0.150
Average ICU mobility scale score	0.7 [0.1-0.9]	0.106	0.590	0.163	0.506

Mean ± standard deviation; Median [interquartile range]

CRRT, continuous renal replacement therapy; ECMO, extracorporeal membrane oxygenation; EN, enteral nutrition; MV, mechanically ventilated; PN, parenteral nutrition; SMA, skeletal muscle area; SMD, skeletal muscle density

[a]using documented weight on admission, with adjusted body weight for BMI >30kg/m^2

[b]Pearson correlation used for normally distributed data, Spearman correlation used for non-normally distributed data

**correlation is significant at the 0.01 level (2-tailed), *correlation is significant at the 0.05 level (2-tailed)

6.4 Conclusion

This chapter provides insight into the short-term precision of CT for muscle assessment in critical illness, suggesting that the method has appropriate precision for this purpose. It also describes, for the first time, the marked losses in CT-measured skeletal muscle CSA at the abdominal area that occur over the first month of critical illness, plateauing at ICU weeks 5 to 7. It also reports that the administration of insulin and sedatives were associated with greater muscle loss, but energy and protein delivery and adequacy were not.

With the observation of significant muscle loss, not only over the first few weeks of ICU admission but up to a month after, this study highlights the critical need for future research to evaluate interventions aimed to attenuate these losses and reverse them during recovery and rehabilitation. Another important finding from this study is the challenges with performing adequately powered studies using CT scans to longitudinally measure changes in muscle health in critical illness. Although this study went 10 years back through the medical record there was only a modest number of patients who met the inclusion criteria and were included in the final cohort. This emphasises the need for future studies aiming to investigate the role of nutrition on muscle changes to use easily applied bedside methods (like those discussed in Chapter 3 and 4) to recruit larger and more heterogenous study populations.

The next chapter discusses and draws the results of chapters 3 to 6 together and discusses the clinical implications and future directions for research in this field.

Chapter 7

Conclusion and Future Directions

This chapter summarises the key findings from the research studies presented in this thesis and outlines the contribution, strengths, and limitations of the research. The implications for future research and clinical practice are also discussed.

7.1 Summary of key findings and contribution to the literature

The studies in this thesis encompass a prospective observational study, a systematic literature review, and a retrospective study, each of which was informed by critical review of the literature as presented in Chapter 1. Firstly, this thesis progresses the knowledge of the importance and role of body composition and muscle assessment in critical illness. Secondly, this thesis explores the relationship between energy and protein delivery and skeletal muscle mass changes in critical illness and describes changes in skeletal muscle mass and quality across different weeks of critical illness. The main findings of this thesis are summarised and discussed below.

1. *Ultrasound-derived muscle thickness at the mid-upper arm and bilateral thighs was strongly associated with CT-measured muscularity at ICU admission.*

At the time of study development in 2015-2016, only one study (VALIDUM study) had reported an evaluation of muscle mass assessed by ultrasound (at the bilateral thighs) compared with a reference method (CT image analysis at the L3 area) at ICU admission[74]. That multi-centre study reported a moderate correlation between the two methods, finding that variance in ultrasound assessment at the thighs alone was able to account for 20% of the variance in CT muscle CSA (R^2 =0.20, P <0.001). The prospective observational study presented in **Chapter 3** (ICU-Muscle), was the first study to evaluate the relationship between muscularity assessed by a multi-site ultrasound protocol and CT-measured

muscularity, finding that the ultrasound measurement protocol with the strongest correlation with CT muscle CSA included assessment at both the mid-upper arm and bilateral thighs. This protocol on its own was able to account for 66% of CT muscle CSA (Adjusted R^2 = 0.66, *P* <0.001), a much stronger relationship than reported in the earlier VALIDUM study. The addition of other routinely available baseline variables (age, sex, and co-morbidity index) further strengthened the association (adjusted R^2 = 0.70, *P* <0.001). This novel finding, published in 2020, indicates that when using ultrasound in the ICU setting, an assessment of muscle thickness at the mid-upper arm and thighs may be a closer reflection of whole-body muscularity than a measurement of the thighs alone, a finding which has also been replicated in healthy volunteers[85]. This discovery is an important step in the progression toward using bedside ultrasound for muscle mass assessment in clinical practice.

2. *A fluid-adjusted fat-free mass variable, derived from bioimpedance spectroscopy (BIS) had good ability to identify those patients with low CT-measured muscularity.*

At the time of conceptualising the ICU-Muscle study, there was one known published study that had compared a bioimpedance variable (phase angle) to CT muscle CSA at L3 at ICU admission[110]. Since that time, there have been two published prospective observational studies that compared bioimpedance variables – FFM and phase angle (one using single-frequency and the other multi-frequency BIA) with CT muscle CSA at L3 at ICU admission[115,116]. Both studies found that FFM measured using bioimpedance analysis significantly overestimated skeletal muscle mass, with increasing disagreement at higher muscle mass values. These findings are consistent with the unadjusted analyses in Chapter 4 and suggest that raw BIS-derived FFM estimates are likely influenced by hydration

status, ethnicity, and the equations used to derive the fat-free mass values, and therefore caution should be exercised when interpreting data unadjusted for hydration status.

As highlighted in **Chapter 4**, the methodology employed in the ICU-Muscle study around the interpretation of BIS data is a strength of this thesis and one that is to date unique in the critical care literature. Firstly, standardised analytical methodology was used to identify and exclude measurements with extreme and unphysiological bioimpedance variables (e.g. as a result of extreme fluid overload) and/or potential measurement errors (e.g. inadequate limb separation, interference with other bedside machinery). Secondly, in measurements remaining in the dataset, an adjustment was made for overhydration using the Chamney model. These findings contribute to the literature with respect to understanding the capabilities of BIS to provide more specific estimates of muscle, when accounting for patients with extreme fluid overload. They also importantly highlight that FFM depletion may be masked using standard bioimpedance techniques if not adjusted for fluid status. BIS-derived measurements of FFM, when adjusted for fluid status using approaches such as Chamney modelling (as shown in **Chapter 4**), also show potential to use body composition-based approaches in clinical practice for the diagnosis of malnutrition. It is possible this approach could improve on the diagnosis of malnutrition (and hence more targeted nutrition delivery) in critical illness and other populations with fluid-overload compared to simpler, but less accurate, single-frequency BIA-derived FFM cut-points.

3. *Subjective physical assessment and arm anthropometry were not able to readily detect low CT-measured muscularity at ICU admission.*

At the time of designing the ICU-Muscle study, there was one known published study comparing nutritional status assessed by the Subjective Global Assessment (SGA) to CT-measured muscle status (normal or low)[127]. It found that when the SGA was not able to readily detect patients with low CT muscularity (with only 37% patients who had low CT muscularity classified as malnourished when using SGA). Misclassified individuals were predominantly male, of a minority ethnic group, and/or overweight and obese. **Chapter 4** presents similar findings, with only 28% of patients with low CT muscularity being identified by the subjective physical assessment section of the SGA tool at ICU admission. To the candidate's knowledge there were no previous studies comparing muscle status assessed by arm anthropometry to a reference method in critical illness. Similarly, to subjective physical assessment, **Chapter 4** reports that only 31% of patients with low CT muscularity were identified as muscle deplete when using arm anthropometry.

The research in this thesis highlights the limitations with using these traditional assessment methods (arm anthropometry and subjective physical assessment) in the ICU setting where oedema may mask muscle depletion and further confirms the need to evaluate other methods such as ultrasound and BIS to provide more accurate assessment of muscularity in critical illness.

4. *There was no clearly detectable association between skeletal muscle mass changes and energy and protein delivery in critical illness.*

The systematic literature review in this thesis was the first to systematically search for studies investigating the association between energy and protein delivery and skeletal

muscle mass changes in critical illness (**Chapter 5**). The original publication, as well as the recent re-run of the search, found no clearly detectable relationship between nutrition delivery and skeletal muscle mass changes in critical illness. The original published review highlighted gaps in the literature and the need for randomised control trials which are not only powered to detect clinically worthwhile changes in muscle mass but also aim to provide a longer period of nutrition delivery (to get clinically significant separation between groups). The re-run of the review also highlighted the evolution of methods to measure muscularity, with a number of recent studies using ultrasound at the quadriceps to investigate the role of nutrition in muscle changes. However, the additional body of evidence did not change the conclusion of the original systematic review, with no clearly detectable association between energy and protein delivery and skeletal muscle mass changes in acute critical illness.

Similarly, the retrospective study presented in **Chapter 6** did not find any association between energy and protein delivery or adequacy (by comparison with dietitian estimates) and skeletal muscle changes in the cohort of patients who had two or more CT scans during ICU admission. However, despite inclusion of all eligible data over a ten-year period, the study was underpowered. Furthermore, the cohort was relatively well fed (via enteral and parenteral nutrition) so there may not have been a sufficient spread of nutrition adequacy needed to demonstrate a link (i.e. due to not being able to identify the impact of severe nutritional deficits on muscle mass).

Taking into account the systematic literature review and the results presented in Chapter 6, it is plausible that a nutrition intervention alone, delivered in the first week or so of critical illness may not be sufficient to attenuate muscle wasting in the context of

significant hypoxia, inflammation, and mitochondrial dysfunction that occurs in acute critical illness[26]. As discussed in more detail in section 7.3 below, future studies aimed at attenuating muscle loss during critical illness should focus on nutrition interventions delivered over a longer period of time, extending to the recovery phase following critical illness and include sequential measurements of muscularity. Furthermore, studies combining nutrition delivery with other anabolic strategies (e.g. physical therapy) are also required.

5. *CT image analysis had good short-term precision to detect changes in skeletal muscle CSA and density at the L3 area in critically ill patients.*

To the candidate's knowledge, the retrospective study presented in **Chapter 6** was the first to investigate the short-term precision of CT image analysis to detect changes in skeletal muscle CSA and density at the L3 area in critical illness. Comparing CT scans performed <24 hours apart, the study found appropriate precision in CSA and density for this purpose. This highlights that if sequential CT scans at the L3 area are performed for diagnostic purposes, then they can be used to assess changes in muscle area and density, both in clinical practice and research.

6. *Using CT image analysis at the L3 area, muscle wasting was marked across the first month of critical illness, plateauing at weeks 5-7. Significant deterioration in muscle quality was also observed over the first few weeks of critical illness. Despite going back 10 years in the medical records, there was only a modest number of patients that had two or more CT scans performed for clinical purposes, highlighting the limitation with relying on CT scans to longitudinally monitor changes in muscularity in critical illness.*

The study presented in **Chapter 6** describes for the first time, changes in CT-measured muscularity across different weeks of critical illness. The findings highlight that muscle wasting can be marked across the first month of critical illness and there is a need for future research to evaluate interventions aimed at attenuating these losses and reversing them during recovery and rehabilitation. The study also emphasised challenges with performing adequately powered studies using CT scans to measure muscularity and the need for future studies using easily applied bedside methods, such as ultrasound and BIS, for muscle assessment in order to feasibly recruit larger and more heterogeneous study populations.

7.2 Thesis strengths and limitations

A strength of this thesis is that a range of different study designs was employed: a prospective observational study, a systematic review, and a retrospective observational study. Strengths of the prospective observational study (ICU-Muscle) include the evaluation of multiple readily applied and clinically applicable methods to assess muscularity at the bedside at ICU admission against a reference method for muscle assessment (CT image analysis). For many years it has been very difficult to undertake quantitative body composition assessments in critically ill patients, and evaluating methods like ultrasound and BIS will ultimately contribute to overcoming the barriers associated with techniques like DXA scanning (transport, positioning) and cost and exposure to ionising radiation (CT Scanning). Additional strengths include the range of intra- and inter-rater reliability tests performed for both CT and ultrasound and the high acquisition rate for ultrasound of the upper arm and bilateral thighs even in a cohort largely composed of trauma patients, demonstrating its feasibility as a bedside body

composition method at ICU admission. In terms of the BIS data, as highlighted earlier (section 7.1, point 2) and in **Chapter 4,** the standardised approach and interpretation of the BIS data to account for overhydration is also strength of the study.

Another strength of the thesis is the systematic literature review presented in **Chapter 5**. The protocol for the review was registered *a priori* and used consistent and objective appraisal tools to critically appraise the literature. The original literature review highlighted the limitations with the existing evidence base at the time, and was used to inform methodology employed in the retrospective study in this thesis. The re-run of the search used the same methodology, therefore its conclusion being similar to the original review is robust. This protocol can be used in a future systematic review on this topic as more literature emerges.

A strength of the retrospective study utilising CT scans to measure changes in muscle mass and quality (**Chapter 6**), was the novel approach to assess the short-term precision of the method by selecting a small number of patients who had CT scans performed <24hours apart (a period where significant changes should not be expected to occur). The finding that there was minimal difference between the scans performed close together, indicates that the changes in CT-measured muscularity over the longer intervals were likely to reflect real changes not measurement variability.

As with all research, there are also limitations to the studies presented in this thesis. Critically ill patients are inherently difficult to study (especially at ICU admission), due to the acuity of illness, the number of investigations and procedures that occur early in critical illness, and the challenges with obtaining informed consent (often from the

medical treatment decision maker/next of kin) at what is often a stressful time. Due to anticipated recruitment challenges, the main prospective study (ICU-Muscle) was designed as a pilot study, to explore the relationship between bedside assessment of muscularity compared to a reference method, while keeping within candidature timelines. As such, the sample size is modest and caution should be exercised when generalising the results (presented in **Chapters 3 and 4**) to the broader ICU population, given the high representation of trauma patients in the sample (due to the inclusion requirement for patients having a CT scan and casemix of the hospital). Furthermore, no morbidly obese patients with a body mass index of >40kg/m^2 were included because of the anticipated issues with visualising bone for the ultrasound muscle thickness measurement, which limits the applicability of the results to this cohort of patients. Another important consideration is that it remains unconfirmed whether CT muscle CSA determined by a single slice at the L3 area is sufficiently representative of whole-body muscle in patients admitted to the ICU.

There are also not yet any pre-published prediction equations to provide estimates of FFM from a CT muscle cross-sectional area from a single CT slice or ultrasound muscle thicknesses to enable full assessment of the accuracy of methods for muscle variables like FFM using techniques like BIS and ultrasound. The small sample size of ICU-Muscle precluded development and validation of equations directly from the dataset in this thesis. However, the methodological and analytical approaches reported in this thesis could be adopted by others for such validation studies. Although the ICU-Muscle study identified the potential for ultrasound and BIS variables to provide a quantifiable estimate of muscularity at ICU admission, this study was not able to investigate the ability for the methods to detect clinically applicable changes in muscularity as no patients had a second

CT scan performed for clinical purposes. Future studies using readily repeated measurements are required for this purpose and are discussed below. Additionally, measurements of muscle function were not feasible in both the ICU-Muscle study (where most participants were intubated and sedated at ICU admission) and retrospective study. As such, investigation of the relationship between muscle mass and muscle function was not possible.

There are also limitations in the application and interpretation of the results of the systematic literature review. The included studies were mostly observational, with small sample sizes, and high risk of confounding. There was also significant heterogeneity within the included studies, in particular there were a range of techniques (CT, BIS, ultrasound, *in vivo* neutron activation analysis) and anatomical locations (biceps, forearm, thigh, abdomen, and whole body) used to track changes in muscularity in response to a nutrition intervention. Therefore, it was not possible to undertake a meta-analysis. The retrospective study also had a modest sample size, and although it was not powered to detect changes in muscularity in relation to energy and protein delivery it provided novel data on muscle changes using a reference method across various weeks of critical illness.

7.3 Implications for future research

The prospective study (ICU-Muscle) in this thesis provides data exploring the utility of several bedside methods to provide an assessment of muscularity in critical illness. These data form a basis for future research informing whether these methods can be routinely implemented into clinical practice, as outlined below.

First, both the 'best-performing' ultrasound protocol and BIS-derived fluid-adjusted FFM variable require external validation, to assess whether the relationship between the methods and CT-measured muscularity holds true in larger and more heterogeneous ICU populations. As previously highlighted, this is challenging in the ICU setting when the reference method is for comparison is most likely to be CT image analysis (due to issues with transporting patients out of the ICU for other specialist body composition analysis, like DXA). CT scanning is costly and involves a significant amount of radiation exposure to the individual. Hence, it is unlikely that many future studies will be able to order single or sequential CT scans specifically for body composition analysis at defined intervals in critically ill patients for research purposes. However, there are opportunities in countries and settings where CT scans are performed more routinely and repeatedly in other patient cohorts not captured in the study presented in this thesis (e.g. in the United Kingdom patients receiving extracorporeal membrane oxygenation routinely have CT scans close to cannulation, where this is not current practice in Australia). Alongside this type of study, it would be ideal to include further inter- and intra-rater reliability studies examining the performance of the methods, and incorporating randomly selected trained individuals to ensure the generalisability of those findings.

Furthermore, it is necessary to investigate how muscle status assessed by ultrasound and BIS at ICU admission (using new and/or existing cut-points) relates to important functional and clinical outcomes following critical illness (e.g. quality of life, return to work, days in hospital, mortality). There is also a need to assess the ability of ultrasound and bioimpedance methods to detect clinically important changes in muscularity over time, and how these changes influence outcomes and/or respond to nutrition or other interventions. This is challenging in the ICU setting. As outlined in **Chapter 6,** there are very few patients who have multiple CT scans performed greater than 7 days apart and as stated above it is unlikely in most settings that CT scans will be performed at pre-determined time points for research purposes or clinical body composition analysis. This means that other ways to determine the ability of the methods to appropriately track changes in muscularity over time need to be employed. This could involve the measurement of muscularity using both ultrasound and BIS together at pre-determined time points, to assess how the measurements compare, and also in relation to an intervention, such as nutrition therapy.

Another important area of research is to evaluate whether the identification of muscle depletion by ultrasound and/or BIS can be used as a surrogate measure for diagnosing malnutrition and/or incorporated into malnutrition diagnostic tools in clinical populations where fluid overload is common. The diagnosis of malnutrition utilising tools such as the Global Leadership Initiative on Malnutrition (GLIM) criteria, requires at least one phenotypic criterion (weight loss, low body mass index, and/or low muscle mass) and one aetiologic criterion (reduced intake, altered absorption, acute and/or chronic inflammation)[62]. Validation of the GLIM criteria is underway internationally, and the global clinical nutrition community has called for prospective and retrospective studies to

do this, including a framework to support the conduct and publication of quality GLIM validation studies[151]. The authors of that paper also specifically recommend that for GLIM, multiple muscle mass measures are used to evaluate which criteria are most associated with clinical outcomes (and enable the appropriate selection of patients who are likely to benefit from nutrition support)[151]. To progress the development and evaluation of ultrasound and BIS to assess muscle mass in clinical settings, both bedside methods can and should be used in such studies - to compare how using these methods impacts the assessment of nutrition status and relationship to corresponding outcomes. Candidate KL is primary investigator on a planned prospective observational study titled *"Prevalence of malnutrition in survivors of critical illness and validation of the new Global Leadership on Malnutrition (GLIM) criteria for diagnosing malnutrition: a prospective observational study".* Within this study muscle status will be determined using both ultrasound (at mid-upper arm and thighs) and BIS (including normally-hydrated FFM variable) and using previously published cut-points to assess and compare their applicability within the GLIM tool at ICU discharge.

Energy targets in critical illness are commonly based on weight-based prediction equations (either fixed prescriptions e.g. kilojoules per kg or gender- and age-based equations), which are known to be inaccurate compared to energy expenditure measured by indirect calorimetry[128]. Because of the issues with weight measurement and extreme fluid shifts in critical illness, an estimated weight is most commonly used for input into these equations. With lean body mass being the biggest driver of metabolic rate, an objective measure/estimate of muscularity may help clinicians better determine nutritional needs of critically ill patients. This is especially relevant in the context of increasing obesity in ICU populations and also where critically ill patients are admitted

with pre-existing chronic illnesses influencing body composition where muscle mass relative to total weight may be low (e.g. chronic lung diseases, elderly patients). Also as highlighted in the introduction (section 1.3) the prevalence of patients with low muscle mass at ICU admission is common (compared to the populations from whom the equations were derived) and if not appropriately identified could lead to overfeeding if nutrition therapy is targeted using weight-based prediction equations. To determine if an objective bedside assessment of muscularity can help predict energy expenditure, studies investigating the relationship between measured energy expenditure and muscle mass measured by ultrasound and/or BIS also warrant further investigation.

In traditional critical care nutrition trials, clinical outcomes such as mortality have been used as the primary outcome measure[150]. However, as mortality rates decrease, it is less likely that a nutrition intervention alone will detectably alter mortality[150]. If there is an impact it may be so small that the sample size required to investigate such an association is likely to be in the tens of thousands. Furthermore, delineating the impacts of nutrition on survival from those of other interventions is very challenging especially in settings where access to the latest evidence-based treatments is high.

This does not mean that nutrition interventions will not be beneficial to patients. In fact, survival at all costs (with permanent disability or significant reduction in quality of life) is not a desirable outcome for most patients[6]. Alternative outcomes that may be important to patients like physical function and strength, reflected by return to normal activities of daily living following hospitalisation are important to investigate[150]. The development and testing of alternate outcomes for critical care nutrition trials, such as skeletal muscle mass

and quality, by non-invasive and easily applied bedside techniques like ultrasound and bioimpedance technology discussed in thesis, are required[152].

As highlighted throughout this thesis, studies aiming to investigate the impact of energy and protein interventions on skeletal muscle changes in critical illness, should be adequately powered. They should also aim to extend the duration of the nutrition intervention and muscle measurements beyond the first few weeks of critical illness and into the recovery phase, which is where nutrition is theoretically more likely to be able to be metabolically processed and result in a positive influence on muscle mass and quality (and corresponding strength and function measures).

7.4 Implications for clinical practice

The prospective study in this thesis, ICU-Muscle (**Chapter 3 and 4**) evaluated methods that could be used by clinicians to objectively measure muscularity and identify patients with depleted muscle mass in the ICU setting. This study identified the potential for a multi-site ultrasound protocol and fluid-adjusted FFM variable to be used for this purpose, but more research is required before they can be routinely recommended for use in clinical practice. Importantly, this thesis has highlighted that those traditionally commonly used methods in clinical practice for muscle assessment; arm anthropometry, and subjective physical assessment, cannot reliably detect individuals with low muscularity at ICU admission. These assessment techniques should be used with caution as they may miss patients who have low muscularity which may result in losing or delaying nutrition support therapy. Evidence from this thesis may help to advocate for an evolution in clinical practice for muscle assessment and the resources to enable this. Furthermore, **Chapter 6** highlighted that although CT image analysis is a reliable and precise method to measure changes in muscle mass and quality in critical illness, it is unlikely to be a useful tool in clinical practice with very few patients in a mixed ICU population having multiple scans for comparison.

The systematic review (**Chapter 5**) and retrospective study (**Chapter 6**) demonstrated that there is no clearly detectable relationship between energy and protein delivery and skeletal muscle mass changes in critical illness. As a result, the data cannot extend or change the current ICU nutrition clinical guideline recommendations about energy and protein targets in critically ill adults.

7.5 Conclusion

The research in this thesis explored the utility of readily applied bedside methods to provide quantifiable assessments of muscularity at ICU admission, finding that ultrasound assessment at the mid-upper arm and bilateral thighs and BIS (using a fluid-adjusted fat-free mass variable) show potential for this purpose. It also highlighted that the other bedside methods; arm anthropometry and subjective physical assessment cannot reliably detect individuals with low CT muscularity at ICU admission and should not be relied upon for this purpose in the ICU setting.

Marked losses of skeletal muscle mass were found over the first month of critical illness, attenuated at weeks 5-7, but no associations with energy and protein delivery were identified. CT image analysis was found to have appropriate short-term precision for detecting changes in skeletal muscle mass and quality in critical illness. Despite the appropriate precision of the method, very few patients had multiple CT scans performed for diagnostic purposes during an ICU admission. This highlights the importance of developing easily applied bedside methods for muscle assessment in the ICU setting where other methods are not possible nor available.

The findings from this thesis have and will form the basis of important ongoing work designed to further evaluate ultrasound and BIS as methods to assess muscularity in critically ill patients, to evaluate its links with outcomes, and to investigate the role of energy and protein delivery and skeletal muscle mass changes in critical illness. There is great potential for these easily applied methods to be used in clinical practice to measure

and monitor muscularity to help target and evaluate nutrition therapy with the overall aim to improve the quality of survival following critical illness.

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Appendices

Appendix 1 Ethics approvals

Chapters 3 and 4 (considered within same ethics approvals)

- Alfred Health Human Research Ethics Committee: Project Number 523/16
- La Trobe University Human Ethics Committee UHEC acceptance for Alfred Health approved project number 523/16

ETHICS COMMITTEE CERTIFICATE OF APPROVAL

This is to certify that

Project No: 523/16

Project Title: Evaluation of bedside methods to measure muscularity in critically ill patients: a prospective observational study

Principal Researcher: Ms Katherine Moore

Protocol Version 9 **dated:** 2-Dec-2016
Participant Information and Consent Form Version 4 **dated:** 30-Oct-2016
Person Responsible Information and Consent Form Version 5 **dated**: 31-Oct-2016
Participant Information and Consent Form (Continuing after Person Responsible consent) Version 2 **dated:** 31-Oct-2016
*was considered by the Ethics Committee on **24-Nov-2016**, meets the requirements of the National Statement on Ethical Conduct in Human Research (2007) and was **APPROVED** on **12-Dec-2016***

It is the Principal Researcher's responsibility to ensure that all researchers associated with this project are aware of the conditions of approval and which documents have been approved.

The Principal Researcher is required to notify the Secretary of the Ethics Committee, via amendment or progress report, of

- Any significant change to the project and the reason for that change, including an indication of ethical implications (if any);
- Serious adverse effects on participants and the action taken to address those effects;
- Any other unforeseen events or unexpected developments that merit notification;
- The inability of the Principal Researcher to continue in that role, or any other change in research personnel involved in the project;
- Any expiry of the insurance coverage provided with respect to sponsored clinical trials and proof of re-insurance;
- A delay of more than 12 months in the commencement of the project; and,
- Termination or closure of the project.

Additionally, the Principal Researcher is required to submit

- A Progress Report on the anniversary of approval and on completion of the project *(forms to be provided)*;

The Ethics Committee may conduct an audit at any time.

All research subject to the Alfred Hospital Ethics Committee review must be conducted in accordance with the National Statement on Ethical Conduct in Human Research (2007).

The Alfred Hospital Ethics Committee is a properly constituted Human Research Ethics Committee in accordance with the National Statement on Ethical Conduct in Human Research (2007).

SPECIAL CONDITIONS
Participant or Person Responsible consent must be obtained for a participant to be enrolled in this study. If, after 3 months, recruitment is not being achieved at a sufficient rate without procedural authorisation, an application may be made for the Ethics Committee to re-consider the use of procedural authorisation (as per S42T of the Guardianship & Administration Act).

SIGNED:

Professor John J. McNeil
Chair, Ethics Committee

Please quote project number and title in all correspondence

LA TROBE UNIVERSITY

University Human Ethics Committee

RESEARCH OFFICE

MEMORANDUM

To: **Ms Katherine Moore, School of Allied Health, College of SHE**

From: **Senior Human Ethics Officer, Ethics and Integrity**

Subject: **UHEC acceptance of The Alfred HREC approved project - 523/16**

Title: **Evaluation of bedside methods to measure muscularity in critically ill patients: a prospective observational study (ICU MUSCLE)**

Date: **16 December 2016**

Thank you for submitting the above protocol to the University Human Ethics Committee (UHEC). Your material was forwarded to the UHEC Chair for consideration. Following evidence of a full review and subsequent final approval by the **The Alfred HREC**, the UHEC Chair agrees that the protocol complies with the National Health and Medical Research Council's *National Statement on Ethical Conduct in Human Research* and is in accordance with La Trobe University's *Human Research Ethics Guidelines*.

Endorsement is given for you to take part in this study in line with the conditions of final approval outlined by **The Alfred HREC.**

Limit of Approval. La Trobe UHEC endorsement is limited strictly to the research protocol as approved by **The Alfred HREC**.

Variation to Project. As a consequence of the previous condition, any subsequent modifications approved by **The Alfred HREC** for the project should be notified formally to the UHEC.

Annual Progress Reports. Copies of all progress reports submitted to **The Alfred HREC** must be forwarded to the UHEC. Failure to submit a progress report will mean that endorsement for your involvement this project will be rescinded. An audit related to your involvement in the study may be conducted by the UHEC at any time.

Final Report. A copy of the final report is to be forwarded to the UHEC within one month of it being submitted to **The Alfred HREC.**

If you have any queries on the information above please e-mail: humanethics@latrobe.edu.au or

contact me by phone.

On behalf of the La Trobe University Human Ethics Committee, best wishes with your research!

Kind regards,

Sara Paradowski
Senior Human Ethics Officer
Executive Officer – University Human Ethics Committee
Ethics and Integrity / Research Office
La Trobe University Bundoora, Victoria 3086
P: (03) 9479 – 1443 / F: (03) 9479 - 1464
http://www.latrobe.edu.au/researchers/ethics/human-ethics

Chapter 6

- Alfred Health Human Research Ethics Committee: Project Number 550/18
- La Trobe University Human Ethics Committee UHEC acceptance for Alfred Health approved project number 550/18

ETHICS COMMITTEE CERTIFICATE OF APPROVAL

This is to certify that

Project No: 550/18

Project Title: Skeletal muscle in the critically ill: application of computed tomography scans to assess changes in muscle mass and quality and the association of energy and protein delivery on these changes

Principal Researcher: Dr Susannah King

was considered for Low Risk Review and ***APPROVED*** *on* **26/10/2018**

It is the Principal Researcher's responsibility to ensure that all researchers associated with this project are aware of the conditions of approval and which documents have been approved.

The Principal Researcher is required to notify the Secretary of the Ethics Committee, via amendment or report, of

- Any significant change to the project and the reason for that change, including an indication of ethical implications (if any);
- Serious adverse effects on participants and the action taken to address those effects;
- Any other unforeseen events or unexpected developments that merit notification;
- The inability of the Principal Researcher to continue in that role, or any other change in research personnel involved in the project;
- A delay of more than 12 months in the commencement of the project; and,
- Termination or closure of the project.

Additionally, the Principal Researcher is required to submit

- A Final Report on completion of the project.

Approval covers the project as described in the application (including any modifications made prior to approval). Low Risk projects are subject to audit and ethical approval may be withdrawn if the project deviates from that proposed and approved.

SPECIAL CONDITIONS

Fully signed Research Collaboration and legal requirements for PhD student working on project to be finalised prior to research research activity commencing at Alfred Health site.

SIGNED:

Professor John J. McNeil
Chair, Ethics Committee

Please quote project number and title in all correspondence

2 May 2019

Research Office

To	Susannah King
From	University Human Ethics Committee
Reference Number	550/18
Project title	Skeletal muscle in the critically ill: application of computed tomography scans to assess changes in muscle mass and quality and the association of energy and protein delivery on these changes
Subject	Externally Approved Project
Date	2 May 2019

The externally approved project submitted above was reviewed and **noted** by the University Human Ethics Committee Chair.

Please note that all requirements and conditions of the original ethical approval for this project still apply.

Should you require any further information, please contact the Human Research Ethics Team on:
T: +61 3 9479 1443| E: humanethics@latrobe.edu.au.

Warm regards,

David Finlay
Chair, University Human Ethics Committee

Appendix 2 Tasks undertaken by the candidate for the original research studies in this thesis

Research task	ICU-Muscle Study	Retrospective study
Study design	√ In conjunction with supervisors and study investigators (80%)	√ In conjunction with supervisors and study investigators (80%)
Ethics application	√ In conjunction with study investigators (80%)	√ In conjunction with study investigators (80%)
Patient screening	√ screening on pre-determined days during recruitment period (90%)	√ screening via medical records (95%)
Consent	√ consented the large majority of study patients (45/50 participants*) (90%)	Not applicable (waiver of consent obtained)
Data collection	√ completed large majority of data collection and participant measurements: • Ultrasound - image acquisition and subjective assessment (45/50 participants*) (90%) • BIS and arm anthropometry (100%) • All other data (participant demographics, fluid balance etc) (90%) • Reliability testing for ultrasound and CT (50% KL and 50% other investigators)	√ completed all data collection and extraction: • CT scan details (administration of contrast, time and date of scan) (100%) • Daily data between two CT scans (including nutrition delivery and adequacy, administration of relevant medications, organ support, ICU mobility score etc) (100%)
Analysis	√ analysed all the CT scans and ultrasound images for muscle assessment (100%) Advice on which scans met criteria for analysis was provided by consultant radiologist (A/Prof Gerard Goh)	√ analysed all the CT scans for muscle assessment (100%) **Note*: Advice about which scans met criteria for analysis was provided by consultant radiologist (A/Prof Gerard Goh)
Statistical analysis	√ ran all the statistical analyses with guidance from study investigators and La Trobe University statistician (70%)	√ ran all the statistical analyses with guidance from study investigators (80%)
Study management and oversight	50% in conjunction with primary supervisor	50% in conjunction with primary supervisor

BIS, bioimpedance spectroscopy; CT, computed tomography

* KL was on maternity leave from mid-July 2017 until March 2018. During this time Dr. Jessica Wang (JW), an ICU registrar and echocardiography fellow at The Alfred hospital screened and recruited five patients into the study

Appendix 3 Abstract submitted to the 2020 ASPEN Nutrition Science and Practice Conference

Can bedside ultrasound be used to assess muscularity on admission to the intensive care unit? A pilot cross-sectional study

Kate J Lambell[1,2], Audrey C Tierney[2,3], Jessica C Wang[4,5], Vinodh Nanjayya[5,6], Adrienne Forsyth[2], Gerard Goh[7], Emma J Ridley[1,6], Selina M Parry[8], Marina Mourtzakis[9] and Susannah J King[1,2]

[1] Nutrition Department, Alfred Health, Melbourne, Australia; [2] Department of Dietetics, Nutrition and Sport, La Trobe University, Melbourne, Australia; [3] School of Allied Health, Faculty of Education and Health Sciences, University of Limerick, Ireland; [4] Department of Critical Care Medicine, University of Calgary, Calgary, Canada; [5] Intensive Care Unit, The Alfred, Melbourne, Australia; [6] Australian and New Zealand Intensive Care Research Centre, Monash University, Melbourne, Australia; [7] Department of Radiology, The Alfred, Melbourne, Australia; [9] Department of Physiotherapy, The University of Melbourne, Melbourne, Australia; [10] Department of Kinesiology, University of Waterloo, Waterloo, Canada

Purpose: The development of bedside methods to assess muscle health is an essential research priority for monitoring nutritional status and predicting functional recovery in critical care[1]. Here, we aimed to compare ultrasound-derived muscle thickness (MT) at five different landmarks with a reference technology (computed tomography (CT) muscle area) at intensive care unit (ICU) admission. Our secondary aims were to 1) evaluate if an ultrasound model, incorporating MT measured at different landmarks and routinely available patient data, could strengthen the relationship with CT muscle area and 2) assess the ability of the optimal ultrasound model to correctly classify patients with low CT muscle area.

Methods: Adult patients who had a CT scan including the third lumbar (L3) area within 72 hours of ICU admission were prospectively recruited. Where possible, MT (cm) was measured at the mid-upper arm, forearm, abdomen, and thighs. CT muscle cross-sectional area (cm^2) at L3 region was assessed using SliceOmatic software. Pearson's correlation compared ultrasound-derived MT and CT muscle area. Linear regression was used to develop prediction models for CT-muscle area based on ultrasound MT measurements. Bland-Altman analyses then compared ultrasound-predicted and CT-measured muscle area. Low CT muscle area was determined using published ICU cut-points (<170cm^2 for men and <110cm^2 for women) . Receiver operator characteristic curve analysis was used to assess the ability of the optimal ultrasound model to correctly classify patients with low CT muscle area. Data are presented as mean ± standard deviation, P<0.05 was considered statistically significant.

Results: Fifty (38 males) ICU patients were enrolled (age 52±20years, BMI 28±5kg/m^2). Patient characteristics are displayed in Table 1. The mean CT muscle area was 173±38 cm^2, with males having significantly higher muscle area compared to females (187±29 cm^2 versus 127±26 cm^2, P<0.001), as did those who were younger (<65 years old) (189±30 cm^2 versus 141±32 cm^2, P<0.001). Ultrasound-derived MT at each anatomical landmark was correlated with CT muscle area (mid-upper arm n=48, r=0.79; forearm n=39, r=0.68; one thigh n=49, r=0.70; bilateral thighs n=49, r=0.75, abdomen n= 39, r=0.68; all P<0.001). The sum of MT at the mid-upper arm and bilateral thighs was the ultrasound combination which had the most complete data (n=47) and a strong positive relationship to CT muscle area (r=0.82, P <0.001) and underwent further evaluation. Incorporating age, sex, and Charlson Comorbidity Index further strengthened the relationship with CT muscle area (r=0.85, P <0.001), and this combination was therefore labeled the optimal ultrasound model. The mean difference between CT-measured and ultrasound-predicted CT muscle area generated from the optimal ultrasound model was -2cm^2 (95% limits of agreement -40 to 36cm^2), with no proportional bias (P =0.102). This model demonstrated good ability to identify the 14 patients with low CT muscle area (area under the curve 0.79).

Conclusion: Ultrasound has the potential to assess muscularity and to identify patients with low muscle mass on ICU admission. Although the results from this study need extension in other settings and tracking over time, we have demonstrated a relationship between muscularity assessed with a widely available and applicable ultrasound method and a reference method. Future research priorities include investigating how muscle status assessed by ultrasound on ICU admission relate to important functional and clinical outcomes (Clinicaltrials.gov NCT03019913).

References:

[1]Arabi, YM et al (2017). *Intensive care medicine*, *43*(9), 1239-1256
[2]Weijs, PJ et al (2014). *Critical care*, *18*(1), R12

Table:

Table 1. Patient clinical and demographic characteristics[a]

Characteristics	All Patients (n=50)
Age category	
<65 years	33 (66)
≥65 years	17 (34)
Sex	
Male	38 (76)
Female	12 (34)
APACHE II	12 [9-16] (2 - 36)
Height (m)	1.72 ± 0.09 (1.50 - 1.98)
Weight (kg)	82 ± 15 (50 - 120)
BMI (kg/m^2)	
Underweight	1 (2)
Normal weight	15 (30)
Overweight	18 (36)
Obese	16 (32)
Charlson Co-morbidity Index	2 ± 2 (0 - 6)
Admission reason	
Trauma	42 (84)
Medical	7 (14)
Surgical	1 (2)
Patients mechanically ventilated	31 (62)
ICU LOS (days)	5 [2-11] (1 – 36)
Hospital LOS (days)	16 [11-24] (3 - 61)
CT to ultrasound protocol (hours)	26 ± 13
ICU admission to ultrasound protocol (hours)	33 ± 12

APACHE, Acute Physiology and Chronic Health Evaluation; BMI, body mass index; ICU, intensive care unit; LOS, length of stay

[a]Values are reported as n; mean±SD(range), median [Q1 to Q3](range), or n(%)

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Chapter 4

The paper from Chapter 4 was published in the *Journal of Human Nutrition and Dietetics* in 2020 and the citation is as follows:

> Lambell KJ, Earthman CP, Tierney AC, Goh GS, Forsyth A, King SJ. How does muscularity assessed by bedside methods compare to computed tomography muscle area at intensive care unit admission? A pilot prospective cross-sectional study. *J Hum Nutr Diet*. 2021 Apr;34(2):345-55. DOI: 10.111/jhn.12804

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Chapter 5

The paper from Chapter 5 was published in the *Journal of Parenteral and Enteral Nutrition* in 2018 and the citation is as follows:

> Lambell KJ, King SJ, Forsyth AK, Tierney AC. Association of energy and protein delivery on skeletal muscle mass changes in critically ill adults: a systematic review. *JPEN*. 2018 Sep;42(7):1112-22. DOI: 10.1002/jpen.1151

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Chapter 6

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Lambell KJ, Goh GS, Tierney AC, Forsyth A, Nanjayya V, Nyulasi I, King SJ. Marked losses of computed tomography–derived skeletal muscle area and density over the first month of a critical illness are not associated with energy and protein delivery. *Nutrition*. 2021. DOI: 10.1016/j.nut.2020.111061

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