The time course of disuse muscle atrophy of the lower limb in health and disease

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Abstract

Short, intermittent episodes of disuse muscle atrophy (DMA) may have negative impact on age related muscle loss. There is evidence of variability in rate of DMA between muscles and over the duration of immobilization. As yet, this is poorly characterized. This review aims to establish and compare the time-course of DMA in immobilized human lower limb muscles in both healthy and critically ill individuals, exploring evidence for an acute phase of DMA and differential rates of atrophy between and muscle groups. MEDLINE, Embase, CINHAL and CENTRAL databases were searched from inception to April 2021 for any study of human lower limb immobilization reporting muscle volume, cross-sectional area (CSA), architecture or lean leg mass over multiple post-immobilization timepoints. Risk of bias was assessed using ROBINS-I. Where possible meta-analysis was performed using a DerSimonian and Laird random effects model with effect sizes reported as mean differences (MD) with 95% confidence intervals (95% CI) at various time-points and a narrative review when meta-analysis was not possible. Twenty-nine studies were included, 12 in healthy volunteers (total n = 140), 18 in patients on an Intensive Therapy Unit (ITU) (total n = 516) and 3 in patients with ankle fracture (total n = 39). The majority of included studies are at moderate risk of bias. Rate of quadriceps atrophy over the first 14 days was significantly greater in the ITU patients (MD -1.01 95% CI -1.32, -0.69), than healthy cohorts (MD -0.12 95% CI -0.49, 0.24) (P < 0.001). Rates of atrophy appeared to vary between muscle groups (greatest in triceps surae (11.2% day 28), followed by quadriceps (9.2% day 28), then hamstrings (6.5% day 28), then foot dorsiflexors (3.2% day 28)). Rates of atrophy appear to decrease over time in healthy quadriceps (6.5% day 14 vs. 9.1% day 28) and triceps surae (7.8% day 14 vs. 11.2% day 28), and ITU quadriceps (13.2% day 7 vs. 28.2% day 14). There appears to be variability in the rate of DMA between muscle groups, and more rapid atrophy during the earliest period of immobilization, indicating different mechanisms being dominant at different timepoints. Rates of atrophy are greater amongst critically unwell patients. Overall evidence is limited, and existing data has wide variability in the measures reported. Further work is required to fully characterize the time course of DMA in both health and disease.

Keywords Muscle; Atrophy; Intensive care; Disuse; Inactivity

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Introduction

Maintenance of an adequate skeletal muscle mass is essential for a healthy, long life. It facilitates independence, locomotion and activities of daily living whilst also playing a key role in glucose homeostasis and the body’s resilience to physiological stress. Low skeletal muscle strength and mass, with or without low physical performance, is known as sarcopenia and has been shown to be associated with an increased risk of falls, fractures, and the need for long term care. It is also associated with the prevalence of long-term health conditions, such as type 2 diabetes, and a worse age-matched risk of mortality.

It is clear that some muscle loss inevitably occurs as part of the innate ageing process, with an average annual loss of 1% of muscle mass and 3% of strength after the age of 70. However, not all older people become sarcopenic, and identification of the underlying factors which drive certain individuals below a critical threshold of muscle mass is therefore a topic of considerable interest. There is evidence of disuse muscle atrophy (DMA) after just a few days of immobilization, and it is now increasingly suggested that intermittent episodes of acute DMA may contribute significantly to the development of sarcopenia. Repeated short periods of immobilization become increasingly common with advancing age due to, for example, ill health, hospitalization, surgical recovery. The loss of muscle function caused by these episodes may lead to a reduction in habitual activity, followed by further episodes of acute DMA and a vicious cycle of punctuated dramatic loss of muscle mass which accelerates the usual age-related changes.

The adverse consequences of acute DMA are not limited to older adults, but also prolong recovery and delay return to normal activity following musculoskeletal injury, illness and surgery in all age groups. Despite this, the majority of research into DMA has been performed in the context of space exploration and consequently many studies report outcomes at only one timepoint and commonly after many weeks or even months of immobilization/disuse. These findings may have little relevance to the effects of shorter periods of immobilization associated with illness, hospitalization and surgery.

Overall, the time course of DMA over shorter periods of immobilization, and how this varies between muscles of the leg is not well characterized. However, what evidence is available from healthy volunteer studies suggests that there may be a differential rate of DMA over the course of immobilization. More rapid loss of muscle mass and function is reported in studies of shorter periods of immobilization, with slower rates seen in prolonged immobilization. This suggests that DMA may slow towards an eventual plateau as immobilization continues, with the most rapid loss of muscle in the initial period of disuse. The rate and extent of DMA also appears to differ between muscles and muscle groups, suggesting that some muscles are more atrophy susceptible (aS) whilst others are more atrophy resistant (aR). Given the catabolic impact of illness, infection and inflammation, these findings are likely to be exaggerated in immobile hospitalized patients, resulting in more rapid and severe DMA than that seen in healthy volunteer studies.

Collectively these findings have many implications. Differential rates of DMA over time suggests different cellular mechanisms and pathways may dominate at different periods, and investigation of mechanisms behind the response of aR and aS muscles to immobilization may yield important insights into the mechanisms through which DMA is controlled and potentially mitigated. From a clinical perspective, if rates of atrophy are greatest at the start of immobilization, early introduction of strategies and therapies to counter this is essential during hospital admissions and other periods of disuse.

Therefore, the primary aim of this systematic review and meta-analysis is to characterize the time-course of DMA in muscles of the human lower limb during immobilization in both healthy and critically ill individuals. Secondary aims include comparison of atrophy rates in healthy and critically ill individuals, comparison of atrophy rates between different muscles and muscle groups of the leg, and exploration of the evidence for non-linear muscle loss, including an acute phase of DMA.

Methods

Study design

This systematic review was registered prospectively with PROSPERO (registration number 106495) and carried out in accordance with the PRISMA statement. Any study reporting data on human lower limb muscle changes during immobilization or admission to ITU over multiple post-immobilization timepoints was included. The minimum outcome reporting required for inclusion was measurement of at least one of (i) muscle volume; (ii) cross-sectional area (CSA); (iii) lean leg mass; or (iv) muscle architecture (muscle thickness, fibre length, pennation angle) at baseline AND at a minimum of two timepoints following immobilization or admission to ITU. Studies which did not report on the above listed measures for leg muscle at multiple timepoints after immobilization or did not involve full immobilization, bedrest, or critical care admission, were excluded.

Literature search

Literature searches were completed by a trained Clinical Research Librarian using the following databases: MEDLINE, Embase, CINHAL and CENTRAL (all searched from their incep-
tion to 01/04/2021). No language, publication type or date restrictions were applied to the searches. Previous systematic reviews of related topics were also searched for relevant studies. References of identified and potentially relevant studies were hand-searched for further relevant studies. Finally, all studies citing the included studies identified on Google Scholar were screened for inclusion. Example search strategies can be found in Appendix S1.

Abstracts were screened independently by two authors (EH and TI) with the aid of Rayyan systematic review software (2016, Qatar Computing Research Institute, Doha, Qatar)27 and were considered for full-text review if either author deemed them to be potentially relevant. A grey literature search as described above was also completed. Full-text versions of all potentially relevant primary studies were then independently screened against the inclusion and exclusion criteria by two authors (EH and TI) and agreement for inclusion reached by consensus.

Data extraction

Study characteristics and outcome data were extracted by one author (EH). Where studies reported the outcomes of interest in graphical form only, relevant data was extracted using WebPlotDigitiser.28 For studies reporting data as percentage change only, attempts were made to contact the authors to get original data. Risk of bias for included studies was assessed using the Risk of Bias In Non-randomized Studies of Interventions (ROBINS-I) assessment tool.29

Statistical analysis

Effect sizes are reported as mean differences (MD) with 95% confidence intervals (CI). Change standard deviations (SD) were calculated by using the baseline SD and final SD and assuming a correlation coefficient of 0.7 using formulae from the Cochrane Handbook. Missing SD values were estimated from other studies. A DerSimonian and Laird random-effects model was used. Statistical heterogeneity was assessed using the $I^2$ statistic. We attempted to conduct tests for publication bias, but the small number of studies precluded this. When studies from different cohorts measured the same outcome at similar timepoints, we performed a subgroup analysis and report the $P$ value from the subgroup differences ($P < 0.05$). All meta-analyses were conducted using Stata Version 16.

Where there was insufficient original data to allow formal comparison, or comparison was not possible due to mathematical constraints (e.g. between % change), pooled means were calculated from original data.

Results

A total of 3702 potentially relevant abstracted were screened for inclusion, of which 3311 were unique. Of the unique abstracts screened, 49 were found to be possibly relevant and underwent full-text review. Following full-text review, a further 14 were excluded, with the remaining 35 studies found to be relevant for inclusion in this review (Figure 1).

Study characteristics

Characteristics of the included studies are shown in Table 1. The date of publication ranged from 1997 to 2020. Thirty-two studies were full-texts, one was a correspondence and two were conference abstracts; all were published in peer-reviewed journals.

Twelve studies reported results from healthy volunteer studies. Of these, three used unilateral lower limb suspension (ULLS) as a means of immobilization, whilst the other nine involved full bed-rest. Eight were cohort studies, and the remaining four consisted of data taken from the control limb of a randomized control trial (RCT). Sample sizes ranged from 6 to 20. Time to first post-immobilization measurement ranged from 2 to 28 days, and time to final measurement ranged from 7 to 88 days.

Eighteen studies reported results from patients admitted to an ITU. Sixteen were cohort studies, with data from the control limb of one case control study and one RCT. Twelve studies contained a mixture of all ITU admissions, two were of patients admitted with traumatic brain injury (TBI), one study was in patients with sepsis, one in patients with respiratory failure, and one in patients having extracorporeal membrane oxygenation (ECMO). Sample sizes ranged from 11 to 100. Time to first post-immobilization measurement ranged from 2 to 10 days, and time to final measurement ranged from 5 to 42 days.

Three studies reported results from patients immobilized with a below knee cast following ankle fracture. All were cohort studies. Sample size ranged from 1 to 20. Time to first post-immobilization measurement ranged from 7 to 14 days, and time to final measurement ranged from 14 to 43 days.

Risk of bias

Risk of bias was assessed using the ROBINS-I tool (Table 2). Overall, 33 studies were found to be at moderate risk of bias, and 2 studies were found to be at low risk of bias. Of those studies found to be at moderate risk of bias, all were at moderate risk in measurement of outcomes due to a lack of blinding in assessors performing or analysing the scans. Three studies performed in ITU patients were at moderate risk of bias due to patient selection, because of varying time from
start of intervention (ITU admission) to baseline scans. Nine studies were at serious risk of bias due to missing data. Eight of these studies were in ITU patients, with a loss of patients as time progressed, and one was in patients following ankle fracture with not all patients attending for scans at all timepoints. One healthy volunteer study was at moderate risk of bias due to deviation from intended intervention, as immobilized patients performed tests of maximum voluntary contraction at 2 timepoints during their immobilization.

**Healthy volunteer studies**

**Whole leg**

One study reporting whole-leg changes as assessed by DXA in 20 immobilized healthy participants showed that lean mass changed by $-3.5\%$ at day 12 (D12), $-5.4\%$ at D31, $-7.0\%$ at D44 and $-8.3\%$ by D56.

**Hip flexors**

One study, in which six healthy young males had 56 days of bed-rest, reported the results of immobilization on the CSA of the iliopsoas. CSA remained stable across all timepoints, with no documented loss seen in either the psoas or iliacus. Compared with baseline measurements iliopsoas CSA decreased by $-2.78\%$ at D14, $-4.86\%$ at D28 and $-2.78\%$ at D42.

**Quadriceps**

Eight studies reported changes in quadriceps muscles during immobilization. Six studies (total 63 participants) used bed rest and two studies (total 30 participants) used unilat-
| Study                  | Type of study/publication | Setting          | Mechanism of immobilization | N  | Muscles studied                          | Imaging modality | Measurements made | Measurement timepoints (day) |
|------------------------|---------------------------|------------------|----------------------------|----|-----------------------------------------|------------------|--------------------|-----------------------------|
| Abe T (1997)           | Cohort. Full paper        | Healthy Bed Rest | 8                          |    | Ant thigh (grouped), Calf (grouped), VL, MG Knee Extensors (grouped), Knee Flexors (Grouped), VL VM, VI, RF, MG, LG, Sol, TA TF, RF | USS              | Muscle Thickness (mm) | 7, 14, 20                 |
| Akima (1997)           | Cohort. Full paper        | Healthy Bed Rest | 10                         |    | Knee Extensors (grouped), Knee Flexors (Grouped), VL VM, VI, RF, MG, LG, Sol, TA TF, RF | MRI              | Vol (cm³), CSA (cm²)  | 10, 20                     |
| Annettan (2017)        | Cohort. Full paper        | ITU (trauma)     | Critical care              | 38 | TA, MG, LG, Sol, Peroneals, Tib Post, FDL, Quads, Hamstring, RF, Vastii, Adductors Quads (grouped), VM, VL, VI, RF | USS | CSA (cm³), MT (cm)  | 5, 10, 15, 20             |
| Armbrecht (2010)       | RCT. Effect of resistive vibration exercise (RVE). Full paper | Healthy Bed Rest | 20                         |    | Whole leg                               | DXA              | Lean Mass (g)       | 12, 31, 44, 55            |
| Belavy (2009)          | RCT. Effect of RVE. Full paper | Healthy Bed Rest | 20                         |    | TA, MG, LG, Sol, Peroneals, Tib Post, FDL, Quads, Hamstring, RF, Vastii, Adductors Quads (grouped), VM, VL, VI, RF | MRI             | Vol (% change)      | 14, 28, 42, 56            |
| Boer (2007)            | Cohort. Full paper        | Healthy ULLS     | 17                         |    | Ant hip muscles, IL, PS, ILP, RF | MRI, USS        | CSA, FL, PA         | 14, 23                     |
| Borges (2019)          | Cohort. Full paper        | ITU (sepsis)     | Critical care              | 37 | RF, VL, VI, RF Quads (grouped)          | USS             | CSA (cm³)           | 2, 4, 6, 10               |
| Cartwright (2012)      | Cohort. Full paper        | ITU (rapsatory failure) | Critical care | 16 | TA, RF | USS | MT (cm) | 3, 7, 10          |
| Dilani (2009)          | Cohort. Full paper        | Healthy Bed Rest | 12                         |    | Ant hip muscles, IL, PS, ILP, RF | MRI             | CSA (cm³)           | 14, 28, 42, 56            |
| Hayes (2018)           | Cohort. Full paper        | ITU (ECMO)       | Critical care              | 25 | RF, VL, VI, RF, Quads (grouped)          | USS | CSA (cm³), MT (cm), Echogenicty CSA (cm²) | 10, 20, CT |
| Hirose (20013)         | Case control vs Electrical muscle stimulation (EMS). Full paper | ITU (CVA/ TBI)  | Critical care              | 11 | Ant Thigh (grouped), Post Thigh (grouped), Ant lower leg (grouped), Post Lower leg (grouped) | CT | 7, 21, 28, 35, 42 |
| Kawashima (2004)       | Cohort. Full paper        | Healthy Bed Rest | 10                         |    | Adductors (grouped), AM, AL, AB Quads (grouped) | MRI             | CSA (cm³)           | 10, 20, RI |
| Kilroe (2020)          | Cohort. Full paper        | Healthy ULLS     | 13                         |    | Qads (grouped) RF                       | MRI             | Vol (cm³)           | 2, 7                      |
| Katani (2018)          | Cohort. Full paper        | ITU (all causes) | Critical care              | 100| RF | USS | MT (cm) | 3, 5, 7, 10, 14, 21 |
| Menids (2009)          | RCT, vs. RVE. Full paper  | Healthy Bed Rest | 6                          |    | iliacus, Psoas, Iliopsoas, Sartorius, RF | MRI | CSA (cm³) | 14, 28, 42, 56    |

(Continues)
eral limb suspension (ULS). One study reported changes in anterior thigh muscle thickness, four reported changes in quadriceps CSA and four reported changes in quadriceps volume. Combined quadriceps muscle volume decreased by $-1.7\%$ at D3, $-5.0\%$ at D7, $-5.71\%$ to $-6.5\%$ by D10 to D14, $-7.31\%$ at D20, $-9.1\%$ by D28, $-12\%$ by

| Study | Type of study/publication | Setting | Mechanism of immobilization | N | Muscles studied | Imaging modality | Measurements made | Measurement timepoints (day) |
|-------|---------------------------|---------|----------------------------|---|----------------|------------------|---------------------|-------------------------------|
| Mikovic (2012) | Cohort. Full paper | Healthy | Bed Rest | 9 | AB, AL, AM, Pec, Gracilis, Sart, RF, Vasti (grouped), MH, LH, EDL, TA, Perineals, FDL, FHL, TP, LG, MG, Sol Quad (grouped) | MRI | Vol (cm$^3$) | 28, 56 |
| Mulder (2006) | RCT, vs RVE. Full paper Cohort. Full paper | Healthy | Bed Rest | 10 | Quad (grouped) | MRI | CSA (cm$^2$)* | Low |
| Nakanishi (2017) | Cohort. Full paper | ITU (all causes) | Critical care | 28 | Quad (grouped) | USS | CSA (% change), MT (% change) | 3, 5, 7 |
| Pardo (2018) | Cohort. Full paper | ITU (grouped) | Critical care | 24 | Quad (grouped) | USS | CSA (% change) | 3, 5, 7, 10, 21 |
| Parry (2015) | Cohort. Full paper | ITU (mixed) | Critical care | 22 | Quad (grouped) | USS | CSA, MT, PA (all % change) | 3, 5, 7, 10 |
| Psatha (2012) | Cohort. Full paper | Ankle Fracture | Critical care | 18 | TA, Sol, GM, GL, Tricep Surae (grouped) | MRI | CSA (% change) | 8, 15, 29, 43 |
| Putuchery (2017) | Cohort. Full paper Correspondence | ITU (all causes) Healthy | Bed Rest | 43 | Calf Muscles (combined) | USS | MT (all % change)* | 7, 10 |
| Rittweger (2005) | Cohort. Full paper | ITU | Critical care | 44 | Bicep, Forearm, Thigh (average of all) | US | CSA (% change)* | 28, 43, 56, 68, 88 |
| Segaran (2017) | Cohort. Full paper | ITU | Critical care | 26 | LG, MG, Sol, Ant lower leg (combined), Post lower leg (combined) | MRI | CSA (cm$^2$) | 7, 14 |
| Seynnes (2008) | Cohort. Full paper | Healthy | ULLS | 8 | Sol, MG, LG | MRI, USS | Vol (cm$^3$), FL, PA MT (cm)* | 14, 28 |
| Silva (2019) | Cohort. Full paper | ITU (TBI) | Critical care | 30 | TA, RF | USS | MT (cm)* | 3, 7, 14 |
| Stevens (2004) | Cohort. Full paper | Ankle fracture | ULLS (below knee cast) | 20 | LG, MG, Sol, Ant lower leg (combined), Post lower leg (combined) | MRI | CSA (cm$^2$) | 7, 14 |
| Shin (2016) | Cohort. Full paper | ITU (all causes) | Critical care | 27 | Upper arm, Thigh, Calf Quads (combined) | Tape | Limb circumference CSA (% change) | 3, 5 |
| ten Haaf (2017) | Cohort. Full paper | ITU (mixed) | Critical care | 14 | Quads (combined) | US | MT (cm)* | 7, 14, 21, 28 |
| Toledo (2017) | Cohort. Abstract only | ITU (all causes) | Critical care | 20 | Quads (combined) | USS | CSA (% change) | 3, 7 |
| Turton (2016) | Cohort. Full paper | ITU (all causes) | Critical care | 22 | MG, VL | USS | MT, FL, PA | 5, 10 |
| Twose (2018) | Cohort. Full paper | ITU (mixed) | Critical care | 26 | RF | USS | CSA (cm$^2$) | 3, 5, 7, 10 |
| Vendenborne (1998) | Cohort. Full paper | Ankle Fracture ITU (all causes) | Critical care | 15 | LG, MG, Sol | MRI | CSA (cm$^2$) | 14, 28 |
| Wapel (2017) | Cohort. Full paper | RCT vs EMS. Abstract only | ITU | 1 | Thigh muscle (combined) | CT | CSA (%change) | 7, 14 |

RCT, randomized controlled trial; RVE, resistive vibration exercise; EMS, electrical muscle stimulation; ITU, intensive treatment unit; ULLS, unilateral lower limb suspension; Quads, quadriceps; VL/M/I, vastus lateralis; medialis; intermedius (respectively); RF, rectus femoris; M/LG, medial/lateral gastrocnemius; Sol, soleus; TA, tibialis anterior; Vol, volume; CSA, cross-sectional area; MT, muscle thickness.

*Data only presented in graphical format and extracted using web plot digitizer.
D42 and −14.4% by D56. Rates of atrophy in individual quadricep muscles varied, with smaller losses observed in rectus femoris (−3.5% to −4.1% at D14 and −5.1% to −7.4% at D56) compared with vastus muscles (−4.7% to −6.7% at D14 and −5.6 to −15.9% at D56).

Combined quadriceps CSA changes correlated with volume changes. There was a decrease of −3.9 to −5.9% at D10–14, −7.6% to −10.0% at D20, −7.6% at D28, −10.3% at D42 and −13.6% by D56. A full breakdown of quadriceps volume and CSA changes are available in Tables S1 and S2. Figure 2A illustrates the time-course of changes in quadriceps muscle volume based on the studies included in this review.31,32,37,38

Anterior thigh muscle thickness, reported by only one study, decreased by −7.1% by D7, −12.6% by D14 and −12.0% by D20.

Hamstrings

Four studies20,32–34 reported the changes observed in knee flexor muscles during immobilization. Three studies (total 39 participants) used bed-rest and one study (total 13 partic-
ipants) used ULS. Two studies reported changes in hamstring CSA,\textsuperscript{20,32,34} whilst all four reported changes in muscle volume.\textsuperscript{20,32–34} Combined hamstring muscle volume decreased by $-1.4\%$ at D3, $-2.1\%$ at D7, $-6.0\%$ to $-6.21\%$ by D10 to D14, $-6.69\%$ at D20, $-6.3\%$ to $-7.19\%$ by D28, $-9.3\%$ by D42 and $-11.3\%$ to $-16.0\%$ by D56. Full details of hamstring volume changes are shown in Table S3. Hamstring CSA decreased by $-1.7\%$ to $-2.38\%$ at D3, $-4.0\%$ to $-5.9\%$ at D7, $-9.3\%$ at D10 and $-9.3\%$ at D20.

Figure 2B illustrates the time-course of changes in hamstring muscle volume based on the studies included in this review.\textsuperscript{20,32–34}

Plantar flexors

Six studies\textsuperscript{20,32,33,36,39,40} reported the changes observed in plantar flexor muscles during immobilization. Five studies (total 55 participants) used bed rest and one study (total 8 participants) used ULS. One study\textsuperscript{36} reported changes in combined calf muscle thickness, and observed decreases of $-7.1\%$ at D7, $-8.2\%$ at D14 and $-6.9\%$ at D28. Four studies reported changes in plantar flexor volume.\textsuperscript{20,32,33,40} Combined plantar flexor muscle volume decreased by $-7.8\%$ at D14, $-11.2\%$ at D28, $-14.4\%$ at D42 and $-18.3\%$ by D56.

Rates of atrophy in individual plantar flexor muscles varied. In general, smaller losses were observed in the lateral belly of the gastrocnemius ($-2.4\%$ to $-7.7\%$ at D14 and $-14.4\%$ to $-16.5\%$ at D56) compared with the medial gastrocnemius and soleus which showed similar rates of atrophy ($-3.0\%$ to $-9.4\%$ at D14 and $-20.4\%$ to $-22.3\%$ at D56). Full results of plantar flexor volume changes are shown in Table S4. Figure 2C illustrates the time-course of changes in plantar flexor muscle volume based on the studies included this review.\textsuperscript{20,32,33,40}

Dorsiflexors

Three studies\textsuperscript{20,32,33} (total 39 participants) reported changes in dorsiflexor muscles during bed-rest immobilization. All three studies reported changes in tibialis anterior volume, with wide variation in results between studies. Decreases in volume ranged from $-0.7\%$ to $-9.2\%$ at D14 and $0.8\%$ to $-7.7\%$ at D28.

Ankle fracture patients

Three studies\textsuperscript{41–43} with a total of 39 patients reported the changes in lower leg musculature during immobilization in plaster cast following ankle fracture. All three studies reported change in CSA of triceps surae muscles, with a decrease of $-6.0\%$ to $-16.0\%$ by D7, $-10.6\%$ to $-26.4\%$ at D14, $-15.5\%$ to $-26.5\%$ by D28, $-13.5\%$ to $-18.9\%$ by D43 and $-32.4\%$ by D56. A full breakdown of changes in plantar flexor CSA in ankle fracture patients is available in Table S5. Figure 3B illustrates the time-course of changes in plantar flexor CSA in ankle fracture patients based on the studies included this review.\textsuperscript{41–43} One study\textsuperscript{41} reported changes in tibialis anterior CSA, with a loss of $-3.08\%$ by D8, $-6.2\%$ at D15, $-10.5\%$ at D28, and $-10.3\%$ by D43.

ITU patients

Eighteen studies reported changes in lower limb muscle size during ITU admission. Thirteen studies reported changes in quadriceps (eight measured CSA\textsuperscript{44–50} and eight measured muscle thickness\textsuperscript{34,46,51–55}), two reported changes in plantar flexors (1 CSA,\textsuperscript{47} 1 muscle thickness\textsuperscript{53}), four reported changes in dorsiflexors (1 CSA,\textsuperscript{47} 1 muscle thickness\textsuperscript{56} and three reported change in hamstring CSA.\textsuperscript{47} Three studies measured combined quadriceps CSA\textsuperscript{47,50} and observed a decrease of $-13.2\%$ by D7 and $-23.9\%$ to $-32.5\%$ on D14. The remaining five studies measured rectus femoris CSA and reported changes of $-1.0\%$ to $-8.7\%$ at D3, $-8.8\%$ to $-13.7\%$ at D5, $-12.5\%$ to $-20.7\%$ by D7, and $-17.7\%$ to $-29.9\%$ by D14. Figure 3A illustrates the time-course of changes in quadriceps muscle CSA in ITU patients based on the studies included this review.\textsuperscript{44–50} A full breakdown of the results from papers included in this study which report changes in muscles of ITU patients is given in Tables S6–S8.
Comparative analysis

Healthy versus ITU

Meta-analysis Meta-analysis for change in rectus femoris CSA after 14 days of immobilization revealed a mean difference (MD) of $-0.12$ (95% CI: $-0.49$ to $0.24$) in healthy participants ($I^2 = 0\%$) (Figure S1), whereas in ITU patients the corresponding MD was $-1.01$ (95% CI: $-1.32$ to $-0.69$) ($I^2 = 84\%$) (Figure S2). On sub-group analysis this indicates a statistically significant greater loss of muscle in ITU patients ($P < 0.001$), although it should be noted that changes in ITU patients were subject to considerable heterogeneity.

Analysis of raw data

To allow an illustrative comparison, pooled means were calculated for quadricep muscle CSA at each timepoint for ITU patients and healthy immobilized volunteers. These results show that ITU patients experienced dramatically more muscle loss than healthy immobilized individuals, with a $-4.6\%$ loss of CSA at D2 (vs. $-1.6\%$), $-13.9\%$ loss at D7 (vs. $-5.6\%$), and $-18.7\%$ at D10–14 (vs. $-5.5\%$). Figure 4 illustrates this difference. 

Healthy versus ankle fracture

There was insufficient original data to allow formal meta-analysis of the difference between muscle loss in immobilized healthy individuals and patients immobilized following ankle fracture. However, the data suggests that muscle loss tends to be greater in patients following ankle fracture than in healthy individuals. Triceps surae muscle volume decreased by $-2.4\%$ to $-9.4\%$ by D14 and $-6.8\%$ to $-17.2\%$ by D28 in healthy participants, whereas muscle CSA decreased by $-10.6\%$ to $-26.4\%$ by D14 and $-15.5\%$ to $-26.5\%$ by D28 in patients following ankle fracture. Comparison of these changes are illustrated in Figure 5.

Comparison between muscle groups

Formal comparison of the rate of muscle atrophy between muscle groups was not possible as many studies reported % change only, with insufficient original data to allow meta-analysis. Pooled means of the change in muscle volume of each muscle group in healthy participants were therefore calculated to allow illustrative comparison. These results show that tibialis anterior had the lowest rates of atrophy with $-1.8\%$ loss at D14 and $-3.2\%$ loss at D28, followed by the hamstring muscles ($-5.3\%$ at D14, $-6.5\%$ D28) and quadri-
Triceps surae showed the greatest losses with $-6.96\%$ loss at D14 and $-11.2\%$ loss at D28. Figure 6 illustrates the difference in rates of muscle atrophy between muscle groups of the lower limb, with full results in Table S9.

Similar trends were seen in patients following ankle fracture. In the one paper reporting two muscles in this patient group, TA CSA reduced by $-3.1\%$ by D8 and $-6.2\%$ by D15, whereas triceps surae reduced by $-6.0\%$ to $-7.2\%$ at D8 and $-10.8\%$ to $-16.7\%$ at D15.

**Time course of disuse muscle atrophy**

There was insufficient original data to allow formal meta-analysis of the difference in rates of atrophy over the time-course of immobilization. Analysis of pooled means revealed that when averaged over D0 to D14, quadriceps muscle volume of healthy volunteers decreased by an average of $-0.46\%/\text{day}$, whereas when averaged from D0 to D28 the rate decreases to $-0.33\%/\text{day}$. Similarly, for the triceps surae of healthy volunteers, the rate of atrophy from D0 to D14 is $-0.50\%/\text{day}$ whereas for D0 to D28 the rate is $-0.40\%$. In the quadriceps of ITU patients the rate of atrophy when averaged over D0 to D2 is $-2.3\%/\text{day}$, from D0 to D7 is $-1.99\%$, and from D0 to D14 $-1.34\%$. These figures suggest that rates of muscle atrophy decrease over time, with more accelerated atrophy in critically unwell patients.

**Measurement method**

Formal comparison of the rate of muscle atrophy as defined by different measurements (muscle thickness vs. CSA vs. volume)
through meta-analysis was not possible due to mathematical constraints, as many studies reported % change only without original measurements, resulting in insufficient comparable original data. Percentage change of the pooled means of original data were therefore calculated to allow an illustrative comparison. In healthy volunteers’ quadriceps muscle volume decreased by −5.6% by D10–14 and −7.4% by D20–28, whereas CSA decreased by −5.5% and −7.9% respectively. In ITU patient’s quadriceps CSA decreased by −18.7% by D10–14, whereas muscle thickness decreased by −20.4%.

Discussion

Despite the large amount of research into skeletal muscle atrophy we have found there is limited evidence to characterize the true time-course of DMA in human lower limb muscles in both healthy volunteers and critically unwell patients, with few studies reporting multiple post-immobilization timepoints. The 12 healthy volunteer studies reported combined results from 140 participants, and the 18 ITU studies a total of 516 patients. Studies varied widely in the muscle groups studied, the measurements used, and the timepoints reported, resulting in limited comparable data. Most studies were at moderate risk of bias, most commonly due to lack of blinding of the assessors. Several ITU studies were also at risk of bias due to high dropout rates as time progressed.

Rates of muscle atrophy

Immobilized healthy muscles start to atrophy quickly, with one study reporting a significant decrease in quadriceps volume after just 2 days. Rates of muscle atrophy are significantly greater in critically unwell patients, with changes in rectus femoris CSA being more than 2.5 times greater in ITU patients than in healthy participants. Whilst this is to be expected due to the presence of potent catabolic drivers such as severe systemic inflammation and starvation in ITU patients, rates of atrophy in patients immobilized following ankle fracture also appear to be greater than in healthy volunteers. Whilst patients following ankle fracture are not subject to the same severe systemic inflammation as ITU patients, localized inflammatory responses may occur, which may contribute to the increased rates of DMA. A further possible explanation is the degree of immobilization that muscles undergo. Following ankle fracture (or ULS for healthy volunteers), legs are totally immobilized in a cast preventing even minimal, non-weight-bearing contractile activity which is possible in bed-rest studies.

Rates of atrophy appear to slow as duration of immobilization progresses. This finding is consistent across all muscle groups and is observed in healthy volunteers, ITU patients and following ankle fracture. This finding is in keeping with observations from other studies, where rates of atrophy follow a pattern of exponential decay with the greatest losses in the first 14 days, slowing over time to reach an eventual nadir. This consistent finding suggests that the mechanisms involved in disuse atrophy may vary over the duration of immobilization and that they are at their most potent in the acute early phase (i.e., day 0 to day 14). It appears that muscle mass maintenance is dependent on continued contractile activity, is lost rapidly upon cessation of this, and rates of atrophy slow once muscle mass is near its intrinsic set point, unless another catabolic factor is present.

Differential atrophy rates between muscles

In ITU patients results from muscle groups other than the quadriceps are limited and no definite variability in rates of atrophy between muscle groups can be identified. However, in healthy volunteers’ triceps surae and quadriceps muscle groups appear to be the most susceptible to disuse, atrophying at around 3-times the rate of ankle dorsiflexors, such as the tibialis anterior, which appear to be the most atrophy resistant. This is in keeping with observations made in other studies, and may reflect a trend towards greater atrophy in those muscle which usually contribute the most force during standing and walking.

There is also some evidence of differential rates of atrophy of individual muscles within a muscle group, although it should be noted that this finding is based on a limited number of studies, with not all studies showing consistent findings. For example, in muscle of healthy volunteers, the vastii of the quadriceps muscle group appears to be more atrophy susceptible than the rectus femoris. These findings mirror those of other studies not included in this review, which report variable rates of muscle atrophy within muscle groups following amputation and tendon rupture. If proven, the notion of differential rates of atrophy in individual muscles within a muscle group raises some questions for the proposition of immobility or inactivity being the main driver behind age-related muscle loss, as the proportion of each individual muscle within the quadriceps seems to be maintained with advancing age.

Formal comparison of changes in different muscle measurements in the assessment of atrophy was outside the scope of this review. However, from the results included in this review, changes in muscle volume and CSA in the quadriceps of healthy volunteers, and CSA and muscle thickness in the rectus femoris of ITU patients, appear to give broadly similar results. This contrasts with the findings of other studies which have suggested that muscle thickness underestimates atrophy in ITU patients when compared with CSA.

As multiple studies have demonstrated that acute DMA is accompanied by a corresponding, but even greater loss of

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muscle function, the findings in this review have important clinical implications, especially as in most patients DMA will be accelerated by inflammation and poor nutrition. Further research is required into the mechanisms at work during the acute phase of DMA and potentially strategies to counteract it. Beyond optimizing patient care to allow and encourage early mobilization, techniques such as bed-based resistance and vibration exercise, and electrical muscle stimulation have shown some promise in the reduction of DMA.

**Limitations**

The major limitation of the current analysis concerns the analysis of raw data from the included studies. Unlike formal meta-analysis, this data does not take account of the fact that individuals within each study are more likely to be similar than those in other studies (patients with sepsis in one study combined with TBI patients) and therefore needs to be interpreted with caution.

Furthermore, despite ultrasound measurements being validated in multiple studies various factors may impact the quality of data acquisition. Whilst it is beyond the scope of this review to analyse the imaging data acquisition techniques of each individual study, we recognize that results based on data obtained without use a gold standard imaging technique must be interpreted with caution.

Finally, as this review intended to characterize the time-course of disuse muscle atrophy in muscles of the human lower limb during immobilization in both healthy and critically ill individuals, we chose to only include studies with measures of muscle mass at multiple timepoints (beyond baseline) to best reflect the true temporality of DMA (versus studies which only report baseline and 1 other timepoint). We acknowledge that this approach will have excluded a number of studies reporting muscle mass losses with disuse; however, alternative recent reviews with a differing search strategy do synthesize the evidence from these (e.g. previous study).

**Conclusion**

In conclusion, further work is required to fully characterize the time course of DMA in the human lower limb in both health and disease. However, results from the studies included in this review suggest that DMA occurs rapidly, with the highest rate of muscle loss in the most acute phase, and that these changes are significantly greater in the critically unwell patient. Both findings highlight the importance of early intervention to minimize muscle loss, especially in unwell patients. Further, rates of DMA appear to vary both between muscle groups and between individual muscles within a muscle group, an observation that must be considered during intervention design.

**Ethical statement**

The authors certify that they comply with the ethical guidelines for authorship and publishing in the Journal of Cachexia, Sarcopenia and Muscle.

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**Conflict of interest**

Edward Hardy, Thomas Inns, Jacob Hatt, Brett Doleman, Joseph Bass, Philip Atherton, Jonathan Lund and Bethan Phillips declare they have no conflicts of interest.

**Online supplementary material**

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

**References**

1. Mitchell WK, Williams J, Atherton P, Larvin M, Lund J, Narici M. Sarcopenia, Dynapenia, and the Impact of Advancing Age on Human Skeletal Muscle Size and Strength; a Quantitative Review. Front Physiol 2012;3:260.

2. Petersen KF, Dufour S, Savage DB, Bilz S, Solomon G, Yonemitsu S, et al. The role of skeletal muscle insulin resistance in the pathogenesis of the metabolic syndrome. Proc Natl Acad Sci U S A 2007;104:12587–12594.

3. Wolfe RR. The underappreciated role of muscle in health and disease. Am J Clin Nutr 2006;84:475–482.

4. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyere O, Cederholm T, et al. Sarcopenia: revised European consensus
on definition and diagnosis. Age Ageing 2019;48:16–31.

5. Deschenes MR. Effects of Aging on Muscle Fibre Type and Size. Sports Med 2004;34:809–824.

6. Srikanthan P, Hevener AL, Karlamangla AS. Sarcopenia Exacerbates Obesity-Associated Insulin Resistance and Dysglycemia: Findings from the National Health and Nutrition Examination Survey III. PLoS ONE 2010;5:e10805.

7. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. Age Ageing 2010;39:412–423.

8. Goodpaster BH, Park SW, Harris TB, Kritchevsky SB, Nevitt M, Schwartz AV, et al. The Loss of Skeletal Muscle Strength, Mass, and Quality in Older Adults: The Health, Aging and Body Composition Study. J Gerontol 2006;61:1059–1064.

9. Narici MV, Maffulli N. Sarcopenia: characteristics, mechanisms and functional significance. Br Med Bull 2010;95:139–159.

10. Dirks ML, Wall BT, Snijders T, Ottenbros CLP, Verdijk LB, van Loon LJ. Neum muscular electrical stimulation prevents muscle disuse atrophy during leg immobilization in humans. Acta Physiol 2014;210:628–641.

11. Oikawa SY, Holloway TM, Phillips AV. The Impact of Step Reduction on Muscle Health in Aging: Protein and Exercise as Countermeasures. Front Nutr 2019;6:75.

12. Järvinen TA, Järvinen M, Kalimo H. Regeneration of injured skeletal muscle after the injury. Muscles Ligaments Tendons J 2013;3:337–345.

13. Lang T, van Loon JIWA, Bloemhof S, Vicò L, Chopard A, Rittweger J, et al. Towards human exploration of space: the THESEx review series on muscle and bone research priorities. NPJ Microgravity 2017;3:8.

14. Padon-Jones D, Sheffield-Moore M, Urban RJ, Sanford AP, Aarsland A, Wolfe RR, et al. Essential amino acid and carbohydrate supplementation ameliorates muscle protein loss in humans during 28 days bedrest. J Clin Endocrinol Metab 2004;89:4351–4358.

15. LeBlanc A, Lin C, Shackelford L, Sinitsyn V, Evans H, Belichenko O, et al. Muscle volume, MRI relaxation times (T2), and body composition after spaceflight. J Appl Physiol 2000;89:2158–2164.

16. Stewart C. • Average length of hospital stay in UK 2010-2017 [Statista. Statista (2019). Available at: https://www.statista.com/statistics/881260/average-length-of-hospital-stay-in-uk/]. (Accessed: 1st March 2020)

17. Aravanis A, Samy EF, Thomas JD, Quirke P, Morris EJA, Finan PJ. A retrospective observational study of length of stay in hospital after colorectal cancer surgery in England (1998-2010). Med 2016;95:e5064.

18. Wall BT, Dirks ML, Snijders T, Senden JMG, Dolmans J, van Loon LIC. Substantial skeletal muscle loss occurs during only 5 days of disuse. Acta Physiol 2014;210:600–611.

19. Rudrappa SS, Wilkinson DJ, Greenhaff PL, Smith K, Idris IJ, Atherton PJ. Human Skeletal Muscle Disuse Atrophy: Effects on Muscle Protein Synthesis, Breakdown, and Insulin Resistance–A Qualitative Review. Front Physiol 2016;7:361.

20. Miokovic T, Armbrrecht G, Felsenberg D, BelavlY DL. Heterogeneous atrophy occurs within individual lower limb muscles during 60 days of bed rest. J Appl Physiol 2012;113:1545–1559.

21. Shackelford LC, LeBlanc AD, Driscoll TB, Evans HJ, Rionan NJ, Smith SM, et al. Resistance exercise as a countermeasure to disuse-induced bone loss. J Appl Physiol 2004;97:119–129.

22. Bass JJ, Hardy EJO, Inns TB, Wilkinson DJ, Piasecki M, Morris RH, et al. Atrophy Resistant vs. Atrophy Susceptible Skeletal Muscles: “aRaaS” as a Novel Experimental Paradigm to Study the Mechanisms of Human Disuse Atrophy. Front Physiol 2021;12:545.

23. Ferrando AA, Sheffield-Moore M, Wolf SE, Herndon DN, Wolfe RR. Testosterone administration in severe burns ameliorates muscle catabolism. Crit Care Med 2001;29:1936–1942.

24. Mukund K, Subramaniam S. Skeletal muscle: A review of molecular structure and function, in health and disease. Wiley Interdiscip Rev Syst Biol Med 2020;12:e1462.

25. Moylan JS, Smith JD, Chambers MA, McLoughlin TJ, Reid MB. TNF induction of atrogin-1/MAFbx mRNA depends on Foxo4 expression but not AKT-Foxo1/3 signaling. Am J Physiol Cell Physiol 2008;295:C986–C993.

26. Page MJ, McKenzie JG, Bossuyt PM, Coutounier C, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an update of the PRISMA statement for reporting systematic reviews. BMJ 2021;372:n71.

27. Ouzzani M, Hammady H, Fedorowicz Z, El-Moore M, Wolf SE, Wilkinson DJ, et al. ROBINS-I: a tool for assessing risk of bias in nonrandomized studies of interventions. BMJ 2016;355:i4919.

28. Armbrrecht G, BelavlY DL, Gast U, Bongrazio M, Touby F, Beller G, et al. Resistive vibration exercise attenuates bone and muscle loss after 90 days’ bed rest and the effects of flywheel resistive exercise and pamidronate: Results from the LTBr study. Bone 2005;36:1019–1029.

29. Rittweger J, Frost HM, Schiessl H, Ohshima H, Allmover B, Treisch P, et al. Muscle atrophy and bone loss after 90 days’ bed rest and the effects of flywheel resistive exercise and pamidronate: Results from the LTBR study. Bone 2005;36:1019–1029.

30. Vandenborne K, Elliott MA, Walter GA, Abdus S, Okereke E, Shaffer M, et al. Extended adaptation strategies to unloading in the human calf muscles. Acta Physiol 2008;193:265–274.

31. Psatha M, Wu Z, Gammie FM, Ratkевич A, Wackerhage H, Lee JH, et al. A longitudinal MRI study of muscle atrophy during lower leg immobilization following ankle fracture. J Magn Reson Imaging 2012;35:686–695.

32. Vandenborne K, Elliott MA, Winter GA, Abdus S, Okereke E, Shaffer M, et al. Longitudinal study of skeletal muscle adaptations during immobilization and rehabilitation. Muscle Nerve 1998;21:1006–1012.

33. Stevens JE, Winter GA, Okereke E, Scarborough MT, EsthEAI JL, George ZS, et al. Muscle adaptations with immobilization and rehabilitation after ankle fracture. Med Sci Sports Exerc 2004;36:1695–1701.

34. Hayes K, Holland AE, Pellegrino VA, Mathur A, Hides JA, Wilson SJ, et al. Acute skeletal muscle wasting and relation to physical function in patients requiring extracorporeal membrane oxygenation (ECMO). J Crit Care 2018;48:1–8.

35. Puthucheary ZA, Rawal J, Michael M, Cavallin B, Ratnayaka G, Chau P, et al. Acute Skeletal Muscle Wasting in Critical Illness. JAMA 2013;310:1591.

36. Parry SM, Puthucheary ZA. The impact of extended bed rest on the musculoskeletal...
system in the critical care environment. 

Extrem Physiol Med 2015;4:16.

47. Hirose T, Shiozaki T, Shimizu K, Mouri T, Noguchi K, Ohnishi M, et al. The effect of electrical muscle stimulation on the prevention of disuse muscle atrophy in patients with consciousness disturbance in the intensive care unit. J Crit Care 2013;28:e1–536.e7.

48. Borges RC, Soriano FG. Association Between Muscle Wasting and Muscle Strength in Patients Who Developed Severe Sepsis And Septic Shock. Shock 2019;51:312–320.

49. Nakashashi N, Oto J, Tsuchumi R, Iuchi M, Onodera M, Nishimura M. Upper and lower limb muscle atrophy in critically ill patients: an observational ultrasonography study. Intensive Care Med 2018;44:263–264.

50. Wappel SR, Bhatti W, Okerere J, Aravagiri A, Ali O, Wells CL, et al. A50. The effect of Neuromuscular Electrical Stimulation on Selected Muscle Groups During Acute Muscle Wasting in Critically Ill Patients Receiving Mechanical Ventilation. Am J Respir Crit Care Med 2018;197.

51. Segaran E, Wandrag L, Stotz M, Terblanche M, Hickson M. Does body mass index impact on muscle wasting and recovery following critical illness? A pilot feasibility observational study. J Hum Nutr Diet 2017;30:227–235.

52. Pardo E, el Behi H, Boizeau P, Verdonk F, Alberti C, Lessot T. Reliability of ultrasound measurements of trans-tibial amputees: an ultrasonographic study. Arch Orthop Trauma Surg 2001;121:307–315.

53. Schmalz T, Blumentritt S, Reimers CD. Selective thigh muscle atrophy in trans-tibial amputees: an ultrasonographic study. Arch Orthop Trauma Surg 2001;121:307–315.

54. Katari Y, Srinivasan R, Arvind P, Hiremathada S. Point-of-Care Ultrasound to Evaluate Thickness of Rectus Femoris, Vastus Intermedius Muscle, and Fat as an Indicator of Muscle and Fat Wasting in Critically Ill Patients in a Multidisciplinary Intensive Care Unit. Indian J Crit Care Med 2018;22:781–788.

55. Toledo D, Freitas B, Carneiro D, Santos D, Dib R, Figueiredo E, et al. Bedside ultrasound muscle layer thickness assessment of the quadriiceps in critically ill patient. Crit Care 2017;21:933.

56. Silva PE, de Cássia Marqueti R, Livino-de-Carvalho K, de Araujo AET, Castro J, da Silva VM, et al. Neuromuscular electrical stimulation in critically ill traumatic brain injury patients attenuates muscle atrophy, neurophysiological disorders, and weakness: a randomized controlled trial. J Intensive Care 2019;7:51.

57. Costamagna D, Costelli P, Sampaolesi M, Penna F. Role of Inflammation in Muscle Homeostasis and Myogenesis. Mediators Inflamm 2015;2015:1–14.

58. van Gassel RJ, Baggerman MR, van de Poll MCG. Metabolic aspects of muscle wasting during critical illness. Curr Opin Clin Nutr Metab Care 2020;23:96–101.

59. Bodine SC. Hibernation: the search for a functional metabolic state in skeletal muscle. Annu Rev Physiol 2006;68:235–256.

60. Shah PK, Stevens JE, Gregory CM, Pathare NC, Jayaraman A, Bickel SC, et al. Lower-Extremity Muscle Cross-Sectional Area After Incomplete Spinal Cord Injury. Arch Phys Med Rehabil 2006;87:772–778.

61. Heikkinen J, Piilonen J, Flinkkilä T, Ohtonen M, Toikka M, et al. Muscle Specificity of Muscle atrophy in patients with consciousness disturbance in the intensive care unit. J Crit Care 2017;21:933.

62. Schmalz T, Blumentritt S, Reimers CD. Selective thigh muscle atrophy in trans-tibial amputees: an ultrasonographic study. Arch Orthop Trauma Surg 2001;121:307–315.

63. Trappe TA, Lindquist DM, Carrithers JA. Muscle-specific atrophy of the quadriceps femoris with aging. J Appl Physiol 2001;90:2070–2074.

64. Paris MT, Day A, Leung R, Watharkar S, Kozar R, Earthman C, et al. Validation of Bedside Ultrasound of Muscle Layer Thickness of the Quadriiceps in the Critically Ill Patient (VALIDUM Study). J Parenter Enteral Nutr 2017;41:171–180.

65. Puthucheary ZA, Mcnelly AS, Rawal J, Connolly B, Sidhu PS, Rowlerson A, et al. Rectus Femoris Cross-Sectional Area and Muscle Layer Thickness: Comparative Markers of Muscle Wasting and Weakness. Am J Respir Crit Care Med 2017;195:136–138.

66. Mueller N, Tainter CR, Lee J, Riddell K, Fintelmann FJ, Grabitz SD, et al. Can Sarcopenia Quantified by Ultrasound of the Rectus Femoris Muscle Predict Adverse Outcome of Surgical Intensive Care Unit Patients as well as Frailty? A Prospective, Observational Cohort Study. Ann Surg 2016;264:1116–1124.

67. Konda NN, Karri RS, Winnard A, Nasser M, Evetts S, Boudreau E, et al. A comparison of exercise interventions for bed rest studies for the prevention of musculoskeletal loss. npj Microgravity 2019;5:12.

68. Kourkoutis K, Tsologlidou A, Kourkouta L. Muscle atrophy in intensive care unit patients. Acta Inform Med 2014;22:406–410.

69. Bunnell A, Nye J, Gellhorn A, Hough CL. Quantitative neuromuscular ultrasound in intensive care unit-acquired weakness: A systematic review. Muscle Nerve 2015;52:701–708.

70. Weinle LM, Summers MJ, Chapple L-A. Ultrasound to measure quadriceps muscle in critically ill patients: A literature review of reported methodologies. Anaesth Intensive Care 2019;47:423–434.

71. Mountzakis M, Parry S, Connolly B, Puthucheary Z. Skeletal Muscle Ultrasound in Critical Care: A Tool in Need of Translation. Ann Am Thorac Soc 2017;14:1495–1503.

72. Marusic U, Narici M, Simunic B, Piotr R, Ritzmann R. Nonuniform loss of muscle strength and atrophy during bed rest: a systematic review. J Appl Physiol 2021;131:194–206.

73. von Haehling S, Morley JE, Coats AJS, Anker SD. Ethical guidelines for publishing in the Journal of Cachexia, Sarcopenia and Muscle: update 2021. J Cachexia Sarcopenia Muscle 2021;12:2259–2261.