


Non-volitional assessment of tibialis anterior force and architecture during critical illness

Bronwen Connolly PhD¹ , Matthew Maddocks PhD², Victoria MacBean PhD¹, William Bernal PhD³,
Nicholas Hart PhD³, Philip Hopkins PhD⁴, Gerrard F Rafferty PhD¹

¹King's College London, Division of Asthma, Allergy and Lung Biology, Department of Respiratory Medicine, UK, ²King's College London, Department of Palliative Care, Cicely Saunders Institute, UK,

³Institute of Liver Studies, King's College Hospital, London, UK, ³Lane Fox Respiratory Unit, Guy's & St. Thomas' NHS Foundation Trust, London, UK, ⁴Department of Critical Care, King's College Hospital, London, UK

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Corresponding author for reprints

Dr Gerrard F Rafferty

Department of Respiratory Medicine

Second Floor, Cheyne Wing

King's College Hospital

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Denmark Hill

London, SE5 9RS

Email: Gerrard.rafferty@kcl.ac.uk

Tel: +44 20 3299 2082

Fax: +44 203 299 3791

Running title

Skeletal muscle in critical illness

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Disclosure of Conflicts of Interest

None of the authors has any conflict of interest to disclose.

Keywords

Critical illness, muscle force, architecture, ultrasound, assessment

ABSTRACT**Non-volitional assessment of tibialis anterior force and architecture during critical illness****Introduction**

Contemporaneous measures of muscle architecture and force have not previously been conducted during critical illness to examine their relationship with intensive care unit (ICU)-acquired weakness.

Methods

Ankle dorsiflexor muscle force (ADMF) using high frequency electrical peroneal nerve stimulation and skeletal muscle architecture via ultrasound were measured in 21 of adult, critically ill patients, 16 at ICU admission.

Results

Thirteen patients were measured on two occasions. Of these 10 measured at ICU admission demonstrated muscle weakness. Despite significant reductions in tibialis anterior (TA_{CSA} , $\Delta = -88.5 \pm 78.8 \text{ mm}^2$, $p = 0.002$) and rectus femoris (RF_{CSA} , $\Delta = -126.1 \pm 129.1 \text{ mm}^2$, $p = 0.006$) cross-sectional area between occasions, ADMF did not change (100HzAD (9.8 (8.0-14.4)kg vs. 8.6 (6.7-19.2)kg, $p = 0.9$).

Discussion

Muscle weakness was evident at ICU admission. No further decrements were observed seven days later despite significant reductions in muscle size. These data suggest that not all ICU weakness is truly 'acquired', and questions our understanding of muscle function during critical illness.

Keywords

Critical illness, muscle force, architecture, ultrasound, assessment

INTRODUCTION

Peripheral skeletal muscle wasting is an acute insult of critical illness¹, and may contribute to the development of intensive care unit-acquired weakness (ICU-AW). Recent data from a longitudinal prospective cohort study of survivors of acute respiratory distress syndrome demonstrated that patients with ongoing ICU-AW at hospital discharge had worse 5 year survival than those without weakness.² Ultrasound has increasingly been used to assess muscle function by measuring parameters of muscle architecture such as cross-sectional area (CSA) and echogenicity.³ Sequential measurements of CSA have previously demonstrated a reduction of almost 20% in muscle bulk in critically ill patients during the first ten days of ICU admission.¹ Furthermore, when comparing ultrasound-derived echogenicity data (a machine-generated parameter⁴) to histological specimens, echogenicity was reported to have a predictive capacity for development of myofiber necrosis⁵, a clinically-relevant finding.

Such measurements of peripheral skeletal muscle architecture do not, however, provide information on contractile properties and force-generating capacity of muscle. Volitional manual muscle testing is restricted to patients sufficiently conscious to perform testing and cannot distinguish true loss of muscle strength from poor motivation and impaired cognition.^{6,7} In contrast non-volitional techniques involving electrical or magnetic motor nerve stimulation eliminate the limitations imposed by patient cooperation, motivation and the clinical environment. Non-volitional measurements of muscle contractility in critically ill patients have been reported for adductor pollicis⁸⁻¹⁰, tibialis anterior^{11,12}, and quadriceps¹³ muscle groups albeit acquired using either the single twitch response or trains of up to four stimuli and therefore not reflecting true muscle strength. 100Hz electrical stimulation of the peroneal nerve is tolerable in healthy subjects and patients with chronic obstructive pulmonary disease (COPD)^{14,15} and, unlike other non-volitional techniques^{8,11,12,9,10,13} the evoked tetanic contraction force reflects true muscle strength, being equal to that

obtained during a maximal voluntary contraction.^{16,15} In addition, assessment of ankle dorsiflexor muscle force (ADMF) is minimally disruptive to patient position or treatment and therefore relatively easy to apply in the ICU environment.

The aim of this study was to measure the degree and trajectory of change in peripheral skeletal muscle strength in patients admitted to ICU by measuring ADMF, and its relationship with contemporaneous assessment of peripheral skeletal muscle (tibialis anterior) architecture.

METHODS

Ethics Approval

Ethical approval was granted (National Research Ethics Committee London – Camden & Islington, 13/LO/02630) and the study conformed to the standards set by the Declaration of Helsinki (1996). Written informed assent for participation was obtained from all participants' nominated next of kin or medical consultant.

Study design and setting

The study was undertaken in three tertiary referral ICUs (Liver, Medical, Surgical) of a university teaching hospital. Measurements were performed in patients on two occasions, separated by seven days.

Participants

Adult (≥ 18 years) patients admitted to ICU and likely to require invasive mechanical ventilation for at least 48 hours and remain in the ICU for 7 days, were eligible for inclusion. Patients could be recruited at any point during ICU admission. Exclusion criteria included acute neurological diagnoses, chronic neuro- or myo-pathies, receipt of neuromuscular blocking agents, trauma injuries (precluding lower limb strength assessment) and pregnancy. The right leg was assessed unless

impaired by clinical factors at the time of initial testing. The same leg was used for reassessment. All patients received standard physical therapy during ICU admission, at the autonomous discretion of their treating therapist. Typically this involved functional mobilisation activities; electrical muscle stimulation and in-bed cycle ergometry were not employed.

Ankle dorsiflexor muscle force

The equipment has been described previously^{14,15}; for full details see the Online Supplement (Section E1). Briefly, ADMF was measured using a bespoke testing device into which the distal lower leg was secured. ADMF elicited by supramaximal electrical stimulation of the common peroneal nerve was measured using a strain gauge attached to the underside of the device footplate. Force response to single twitch (TwAD) and 1second trains of electrical stimulation at 5, 10, 20, 30, 40, 50, 80 and 100Hz (100HzAD) were recorded. Stimulation at half maximal tetanic tension and the maximal relaxation rate (MRR) of the twitch response were determined. The mean±SD between-occasion coefficients of variation for TwAD and 100HzAD were 6.5±1.7% and 3.5±0.7% respectively.

Ultrasound imaging of peripheral skeletal muscle

Tibialis anterior (TA) and quadriceps rectus femoris (RF) muscle cross-sectional area, pennation angle, echogenicity and subcutaneous thickness were assessed by an experienced investigator (BC) using real-time B-mode ultrasound^{14,15}. Ultrasound imaging techniques are summarised below with full details in the Online Supplement (Section E2)

Tibialis anterior

Images of TA cross-sectional area (TA_{CSA}), pennation angle (TA_{PA}), echogenicity (TA_{EI}) and subcutaneous thickness ($TA_{S_{thickness}}$) were acquired using a 2-6MHz curvilinear and 4-12MHz linear (pennation angle only) transducer (C6-2 and L12-4 respectively, Philips Sparq Ultrasound System, Philips Healthcare, MA, USA) from the superior aspect of the distal lower limb at a point one-third of

the distance from the tibial plateau to the lateral malleolus, with the participant semi-supine, the knee supported in passive extension and the foot supported in neutral¹⁷⁻¹⁹. Oblique cross-sectional imaging was minimised by the operator using visual inspection to obtain the smallest cross-sectional area. Scanning depth of TA was set to where the deep margin of the muscle could be visualized between the tibia and fibula.

Quadriceps rectus femoris

Imaging of RF muscle architecture (RF_{CSA} , RF_{El} , RF_{PA} , $RF_{Sthickness}$) was performed in a similar manner (Online Supplement, Section E2), with the quadriceps muscle measured at a point two-thirds of the distance from the anterior superior iliac spine and the superior patellar border. Visualisation of the femur was used to assist scanning depth. RF_{PA} was imaged with the probe rotated 90°, and the average value from five individual fibre angles determined.

STATISTICS

Data were tested for normality and expressed as either mean±SD or median (IQR). Differences in ADMF and architecture parameters between testing occasions were determined with (Wilcoxon signed-rank tests.). Predicted values and cut-offs for 100HzAD were determined from a regression model derived in similarly aged healthy controls¹⁴: $100HzAD(kg) = 4.431 + -0.116age + 0.030 TA_{CSA} - 0.389$ if female. Muscle weakness in critically ill patients was defined as 1.645 residual standard deviations below the predicted value derived from 100HzAD responses from healthy subjects. Thresholds of losses in cross-sectional area exceeding 9.24%²⁰ and 3.48%¹⁸ were used to define muscle wasting in RF and TA muscles respectively. Relationships between ADMF and TA muscle architecture were assessed using Spearman's correlation analysis. A p value less than 0.05 was considered statistically significant. Data were analysed using SPSS (SPSS Inc, Chicago, IL, US) and GraphPad Prism (v6 for Windows, GraphPad, La Jolla, CA, US).

RESULTS

Participants

Twenty-five patients were recruited between June 2013 and July 2014. Three patients were unable to undergo testing due to clinical deterioration between recruitment and testing (n=1 death, n=1 post-tracheostomy procedure, n=1 post-extubation). One further patient was unable to complete assessment due to withdrawal of personal consultee advice. Of the remaining 21 patients enrolled, 16 underwent initial testing within 24 hours of ICU admission. Characteristics of the cohort are presented in Table 1. By the second testing occasion, 3 patients had died, 1 patient refused consent for ongoing participation, and in 4 patients a deterioration in clinical status precluded testing. Overall 13 patients were reassessed on the second occasion (10 (76.9%) with initial testing at ICU admission). All patients were intubated on the first testing occasion. Five of the 13 patients tested on the second occasion had been extubated but remained on the critical care unit.

Ankle dorsiflexor muscle force

No adverse events occurred during testing, and measurement of ADMF was feasible and well tolerated by all patients. In the overall cohort of 21 patients, TwAD and 100HzAD were 3.0(2.2-4.2)kg and 10.5(8.8-15.2)kg respectively. There was no difference in TwAD or 100HzD between patients recruited at or during ICU admission (Table 2). In the 13 patients with repeat measurements there was no change in TwAD, 100HzAD, or TwAD and 100HzAD normalised to TA_{CSA} , between testing occasions (Table 3, Figure 1). Complete force-frequency curves were obtained in all patients at initial testing and in 12/13 patients on the second testing occasion when one patient was measured at 100Hz only (Figure 2). Summary values for the whole cohort at initial and second testing occasions are shown in Figure 3. There was no difference in stimulation frequency for half-maximal tetanic contraction or MRR on the first testing occasion compared to the second.

Tibialis anterior and quadriceps rectus femoris muscle architecture

No differences in muscle architecture for TA and RF were observed between patients recruited at or during ICU admission (Table 2). In patients with repeat assessments (Table 3) it was not possible to measure RF muscle architecture in one patient on either day due to obesity, and RF_{PA} could not be accurately measured in four patients. Significant reductions were observed in both TA_{CSA} between testing occasions, equivalent to overall percentage reductions of $-9.6\pm 9.5\%$ and $-17.2\pm 15.5\%$ respectively. Individual trajectories of change for both muscle groups are presented in the Online Supplement (Section E3).

Wasting, as defined above, was observed in 10/12 (83.3%) patients for RF and 10/13 (76.9%) patients for TA muscles between testing occasions. Wasting of both muscle groups was observed in eight patients, isolated wasting of RF in two patients and of TA in one patient. One patient did not demonstrate wasting in either muscle group. ADMF was compared between patients presenting with and without TA muscle wasting. No difference in measures of TwAD or 100Hz AD were evident (Online Supplement, Section E4). Presence of chronic comorbidity also did not differ between those patients with and without TA wasting. Significant reductions were observed in both RF muscle echogenicity and echogenicity standard deviation between testing occasions (Table 3). No other changes RF or TA muscle architecture echogenicity were observed.

Relationships between ADMF and tibialis anterior muscle architecture

Positive correlations were observed between TA_{CSA} and RF_{CSA} on testing occasion 1 (Spearman's $r=0.7$, $p=0.01$) and 2 (Spearman's $r=0.6$, $p=0.045$) (Online Supplement, Section E4). Only 100HzAD and TA_{CSA} demonstrated a significant relationship on the second testing occasion (Spearman's $r=0.6$, $p=0.047$; Online Supplement, Section E5). No other relationships were observed.

Predicted weakness

Using the previously described regression model, predicted values for 100HzAD were determined in 19 of the 21 patients of the main cohort (n=2 missing TA_{CSA} data); observed 100HzAD was found to be significantly lower than predicted 100HzAD (Table 2), and weakness was evident in 16 (84.2%) patients. A similar pattern was evident when examining the 16 patients from the main cohort who were tested within 24hours of ICU admission. Fourteen patients (same n=2 missing data) had significantly reduced 100HzAD compared to predicted (Table 2), with 12 patients demonstrating weakness.

Predicted 100HzAD values were also determined for the 13 patients assessed on both occasions. ADMF weakness was observed in 12 patients on testing occasion one and in 11 patients on occasion two. On both occasions 100HzAD was significantly lower than predicted

DISCUSSION

In this study involving contemporaneous measures of muscle strength and architecture, significant ankle dorsiflexor muscle weakness was observed in critically ill patients on admission to ICU. Despite significant reductions in TA_{CSA} during the critical illness course, no further decrements in ADMF were observed. Although changes in cross-sectional area were observed in both proximal (RF) and distal (TA) lower limb muscles, only RF showed changes in muscle quality.

Our data suggest antecedent decline in force-generating capacity prior to ICU admission. The reduced strength on ICU admission could therefore reflect a combination of the development of critical illness as well as the presence of chronic comorbidity. This, in combination with absence of further decline in muscle force during the admission period, challenges the commonly accepted term of ICU-*acquired* weakness. Certainly this presentation is a clinical diagnosis of exclusion following treatment of all identifiable causes including the ICU-admission diagnosis.²¹ Nonetheless weakness developing *pre*-ICU admission is also a feature. 'Critical illness-associated weakness' may

be a more reflective term; further investigation in larger cohorts of patients with varying degrees of pre-existing weakness would be valuable. These data also suggest that other mechanisms such as changes within the central nervous system resulting in impaired muscle recruitment and reduced volitional muscle strength could be important in explaining the profound impairment observed in many survivors of critical illness.^{22,23}

We have confirmed previous findings of reduced muscle contractility in sedated and ventilated critically ill patients.^{8,11,12,9,10,13} Longitudinal, as well as cross-sectional, measurements were performed in the current study and most initial testing was undertaken on day one of ICU admission. Such measures allowed not only characterisation of the underlying level of muscle weakness at ICU admission but also the ongoing impact of ICU stay. Our data have demonstrated that the initial insult of critical illness on muscle force occurs early, possibly prior to hospitalisation, and does not continue to the same degree during the illness course. We cannot, however, exclude changes in muscle function that occurred immediately on entry to ICU prior to assessment of skeletal muscle function. We also observed no consistent relationship between peripheral skeletal muscle size and force at either time points of assessment. This could be as a result of a small sample size resulting in type II error, but also indicative of a limitation of the ultrasound technique.

Previous studies from this laboratory and others have demonstrated relationships between muscle size and strength in the knee extensor^{24,25} and ankle dorsiflexor muscles^{14,15} of COPD patients and age-matched control subjects. Nonetheless, muscle CSA does not fully account for variation in muscle strength. Although the current ultrasound findings mirror previously reported declines in such parameters in ICU patients³, ultrasonographic evaluation of muscle architecture solely reflects morphological features. Therefore the effects on muscle CSA of changes in lean muscle mass which are thought to occur during ICU admission could be masked by intrinsic alterations in muscle properties including infiltration of fat, fibrous tissue or extracellular fluid^{26,4,27}. It is possible that

clinical procedures (sedation, neuromuscular blockade), immobilisation and altered fluid balance during the acute phase of critical illness lead to an overestimation of CSA on D1 and would also explain the significant reductions in muscle CSA that were observed between D1 and D7 of admission despite no change in muscle strength. Furthermore this could also explain why a significant relationship between ADMF and TA_{CSA} was only observed at the second assessment, later in the ICU stay.

Despite significant reductions in RF_{CSA} and TA_{CSA} there were no reductions in 100HzAD. Differential atrophy of lower limb muscles has been demonstrated during prolonged bed rest²⁸ and the predominant slow twitch fiber profile of the TA compared to RF²⁹ may result in different responses in muscle architecture to the critical illness process. Such changes do not however explain the absence of change in muscle strength despite reductions in TA_{CSA} . No changes were observed in TA echogenicity, half maximal tetanic tension or MRR between testing occasions, which may have been expected if substantial changes in muscle quality⁴ or muscle fiber composition had occurred.³⁰⁻³² It is also noteworthy that although TwAD was similar to that reported previously by our group for healthy elderly adults of a comparable age¹⁴, TwAD:100HzAD was also increased. Such data highlight the limitations of the 1Hz twitch technique³³⁻³⁵.

Our cohort were a highly selected, non-sequential convenience sample determined by consultant assent and investigator availability. Whilst this limits generalisability, the cohort sizes were larger than any previous studies examining sequential changes in neuromuscular ultrasound properties in critically ill patients^{36,37} or that involved non-volitional assessment of skeletal muscle strength^{12,13,38}. Nonetheless we acknowledge the small sample may yield unexpected findings, and that only approximately two-thirds of the cohort underwent repeat testing. Half of the attrition was, however, due to unmodifiable reasons of patient death or lack of consent. These occurrences, in combination with deteriorating clinical status in some patients, serve to highlight the overall

challenges associated with studying this particular patient group. It is important to note that more than three-quarters of patients in the overall cohort, and those who underwent testing on both occasions, were recruited within 24 hours of ICU admission.

Animal studies have previously shown decrements in force generating capacity following prolonged immobilization³⁹⁻⁴¹. Translating such findings to interpret our data is not straightforward. Unlike studies examining disuse atrophy in models employing healthy animals, the ICU patients studied were not fit and healthy prior to ICU admission and all had multiple comorbidities. The weakness observed at ICU admission most likely reflects the patients' previous physical condition as well as any acute insult of critical illness necessitating ICU admission. Quadriceps contractility has been shown to be four times lower in ICU patients with pre-existing sickness compared to healthy subjects but only two times lower than COPD patients¹³. The subsequent lack of change in skeletal muscle strength may also reflect opposing effects of immobilization, resolution of critical illness, or the influence of physical activity, via voluntary or involuntary movements, or focused physical rehabilitation. The lack of change in 100HzAD may also have been due to a 'floor effect' and the inability of patients to weaken further i.e. given the already reduced baseline muscle strength further weakness was not possible. . This was, however, a relatively small physiological study in a heterogeneous ICU population. Larger studies are therefore highly recommended to validate these findings and examine the magnitude and trajectory of change in muscle strength across differing phenotypes of critically ill patients. Nonetheless these data still question our knowledge around how muscle behaves in the context of critical illness across the spectrum of patients admitted to the ICU, and whether all patients truly 'acquire' weakness in the ICU.

Whilst the current study used the trace method to assess muscle echogenicity, Cartwright *et al*³⁶ defined their ROI as a 2x2cm portion within the muscle, and Grimm *et al*⁴² adopted the Heckmatt grading scale. The trace method was adopted as it offered a standardised approach which reflects

changes across the entirety of muscles evaluated regardless of size and avoids sampling from adjacent muscle groups⁴³, and the influence of ROIs located in different portions of the muscle CSA image.⁴⁴ In the current study, the same experienced investigator (BC) performed all ultrasound measurements. Further data are, however, required to determine the clinical relevance of echogenicity findings e.g. indicating myofiber necrosis⁵ as well as the significance of measures of CSA obtained early in critical illness.

We assessed changes in muscle composition, using echointensity analysis⁴ alongside measurement of CSA as reductions in lean muscle mass could be masked by infiltration of fat, fibrous tissue or extracellular fluid.^{27,4,26} Echointensity findings for TA and RF muscles differed to previously reported studies in critical care patients where changes analogous to mild muscle breakdown and disruption of normal muscle architecture akin to myopathy were noted^{36,42}, albeit these findings required muscle biopsy data for substantiation.

We applied muscle-specific thresholds for determining wasting of both TA¹⁸ and RF²⁰ muscles, albeit only the latter were derived from a critically ill population. Nonetheless this approach reflects a more accurate method for classification, avoiding assumptions that peripheral skeletal muscle groups with differing location (either proximal or distal), fiber type composition and function demonstrate similar rates of wasting. Determining which peripheral muscle group best reflects patient status overall is unclear. Fiber type composition is not uniform across all muscle groups²⁹ and this limits the assumption of extrapolating weakness in one muscle, to be representative of a generalised peripheral skeletal muscle weakness effect. Assessment of quadriceps function, might be considered more 'functionally' relevant¹³, than tibialis anterior however the patient repositioning required to measure quadriceps twitch tension is not unsubstantial in contrast to that required to assess ADMF, which requires minimal patient disturbance. Volitional scores of global muscle strength such as the Medical Research Council Sum-score used to diagnose ICU-AW²¹, also omit key

muscle groups important for upper limb function and dexterity such as the hand muscles⁴⁵. We defined weakness using a regression equation incorporating values of TA_{CSA} , age and sex¹⁴, determined in age-matched healthy subjects. The relationship between muscle cross-sectional area measured using ultrasound and strength has not been extensively investigated in critically ill patients. However we recognise that under such circumstances, the disease pathology, severity of acute illness and influence of various medical interventions may result in the relationships between muscle size and strength observed in healthy subjects no longer being entirely valid in this patient population. We also reported data for healthy individuals to demonstrate acceptable levels of inter-occasion coefficients of variation and infer confidence in the ADMF technique. Acquiring such reliability data (either intra-observer, inter-observer or inter-occasion) may not be feasible in the critically ill patient population, where changes in clinical status would influence the results. This in turn, highlights that variance evident in 100HzAD in the current study must be related to the nature of the clinical condition of patients.

CONCLUSION

Reduced force-generating capacity was evident in critically ill patients even on day one of ICU admission. No further decrements in ADMF were observed after one week despite the presence of significant reductions in TA_{CSA} . These data contribute to our understanding of the individual trajectories of change in peripheral skeletal muscle during critical illness and the potential mechanism underlying such changes. Whilst use of ultrasound offers a clinically useful tool for monitoring parameters of muscle architecture, these data fail to provide a true physiological assessment of the functional status of the muscle.

ABBREVIATIONS LIST

ICU-AW, intensive care unit-acquired weakness; ICU, intensive care unit; COPD, chronic obstructive pulmonary disease; CSA, cross-sectional area; ADMF, ankle dorsiflexor muscle force; TwAD, single

twitch ankle dorsiflexor force; 100HzAD, 100Hz ankle dorsiflexor force; MRR, maximum relaxation rate; TA_{CSA}, tibialis anterior cross-sectional area; RF_{CSA}, rectus femoris cross-sectional area; APACHE, acute physiology and chronic health evaluation; ROI, region of interest

REFERENCES

1. Puthuchery ZA, Rawal J, McPhail M, Connolly B, Ratnayake G, Chan P, et al . Acute skeletal muscle wasting in critical illness. *JAMA* 2013;310(15):1591-1600.
2. Dinglas V, Friedman L, Colantuoni E, Mendez-Tellez P, Shanholtz C, Ciesla N, et al Muscle Weakness and 5-Year Survival in Acute Respiratory Distress Syndrome Survivors*. *Critical Care Medicine* March 2017;45(3):446-453.
3. Connolly B, MacBean V, Crowley C, Lunt A, Moxham J, Rafferty GF, et al . Ultrasound for the Assessment of Peripheral Skeletal Muscle Architecture in Critical Illness: A Systematic Review. *Crit Care Med* 2014;43(4):897-905.
4. Pillen S, van Dijk JP, Weijers G, Raijmann W, de Korte CL, Zwarts MJ. Quantitative gray-scale analysis in skeletal muscle ultrasound: A comparison study of two ultrasound devices. *Muscle Nerve* 2009;39(6):781-786.
5. Puthuchery ZAP, Phadke RF, Rawal JM, McPhail MJWP, Sidhu PSF, Rowleron AP, et al Qualitative Ultrasound in Acute Critical Illness Muscle Wasting. *Crit Care Med* 2015;43(8):1603-1611.
6. Connolly B, Jones G, Curtis A, Murphy P, Douiri A, Hopkinson N, et al Clinical predictive value of manual muscle strength testing during critical illness: an observational cohort study. *Critical Care* 2013;17(5):R229.
7. Hough C, Lieu B, Caldwell E. Manual muscle strength testing of critically ill patients: feasibility and interobserver agreement. *Critical Care* 2011;15(1):R43.
8. Eikermann M, Koch G, Gerwig M, Ochterbeck C, Beiderlinden M, Koeppen S, et al . Muscle force and fatigue in patients with sepsis and multiorgan failure. *Intensive Care Med* 2006;32(2):251-259.

9. Harris ML, Luo YM, Watson AC, Rafferty GF, Polkey MI, Green M, et al . Adductor pollicis twitch tension assessed by magnetic stimulation of the ulnar nerve. *Am J Respir Crit Care Med* 2000;162(1):240-245.
10. Pickles J, Kaul S, Gouda M, Harris M, Ervine M, Donaldson N, et al Weaning and hand muscle weakness in intensive care. *Am J Respir Crit Care Med* 2004;169:A124.
11. Ginz HF, Iazzo P, Girard T, Urwyler A, Pargger H. Decreased isometric skeletal muscle force in critically ill patients. *Swiss Medical Weekly* 2005;135:555-561.
12. Ginz HF, Iazzo P, Urwyler A, Pargger H. Use of non-invasive-stimulated muscle force assessment in long-term critically ill patients: a future standard in the intensive care unit? . *Acta Anaesthesiol Scand* 2008;52:20-27.
13. Vivodtzev I, Devost AA, Saey D, Villeneuve S, Boilard G, Gagnon P, et al . Severe and early quadriceps weakness in mechanically ventilated patients. *Critical Care* 2014;18(R431).
14. Maddocks M, Jones M, Snell T, Connolly B, de Wolf-Linder S, Moxham J, et al . Ankle dorsiflexor muscle size, composition and force with ageing and chronic obstructive pulmonary disease. *Exp Physiol* 2014;99(8):1078-1088.
15. Seymour JM, Ward K, Raffique A, Steier JS, Sidhu PS, Polkey MI, et al . Quadriceps and ankle dorsiflexor strength in chronic obstructive pulmonary disease. *Muscle Nerve* 2012;46(4):548-554.
16. Bigland-Ritchie B, Jones DA, Woods JJ. Excitation frequency and muscle fatigue: Electrical responses during human voluntary and stimulated contractions. *Exp Neurol* 1979;64(2):414-427.
17. Esformes JI, Narici MV, Maganaris CN. Measurement of human muscle volume using ultrasonography. *European Journal of Applied Physiology* 2002;87(1):90-92.
18. Rankin G, Stokes M. Reliability of assessment tools in rehabilitation: an illustration of appropriate statistical analyses. *Clin Rehabil* 1998;12(3):187-199.
19. Scholten RR, Pillen S, Verrips A, Zwarts MJ. Quantitative ultrasonography of skeletal muscles in children: Normal values. *Muscle Nerve* 2003;27(6):693-698.

20. Bloch SAA, Lee JY, Wort SJ, Polkey MI, Kemp PR, Griffiths MJD. Sustained Elevation of Circulating Growth and Differentiation Factor-15 and a Dynamic Imbalance in Mediators of Muscle Homeostasis Are Associated With the Development of Acute Muscle Wasting Following Cardiac Surgery*. *Crit Care Med* 2013;41(4):982-989.
21. Stevens RD, Marshall SA, Cornblath DR, Hoke A, Needham DM, de Jonghe B, et al . A framework for diagnosing and classifying intensive care unit-acquired weakness. *Crit Care Med* 2009;37(10 Suppl):S299-308.
22. Berg H, Tesch P. Changes in muscle function in response to 10 days of lower limb unloading in humans. *Acta Physiol Scand* 1996;157:63-70.
23. Berg HE, Larsson L, Tesch PA. Lower limb skeletal muscle function after 6 wk of bed rest. *J Appl Physiol* 1997;82(1):182-188.
24. Seymour JM, Ward K, Sidhu PS, Puthuchery Z, Steier J, Jolley CJ, et al Ultrasound measurement of rectus femoris cross-sectional area and the relationship with quadriceps strength in COPD. *Thorax* 2009;64(5):418-423.
25. Shrikrishna D, Patel M, Tanner RJ, Seymour JM, Connolly BA, Puthuchery ZA, et al Quadriceps wasting and physical inactivity in patients with COPD. *Eur Respir J* 2012;40(5):1115-1122.
26. Fukumoto Y, Ikezoe T, Yamada Y, Tsukagoshi R, Nakamura M, Mori N, et al Skeletal muscle quality assessed from echo intensity is associated with muscle strength of middle-aged and elderly persons. *European Journal of Applied Physiology* 2012;112(4):1519-1525.
27. Sipila S, Suominen H. Ultrasound imaging of the quadriceps muscle in elderly athletes and untrained men. *Muscle Nerve* 1991;14(6):527-533.
28. Miokovic T, Armbrecht G, Felsenberg D, Belavý DL. Heterogeneous atrophy occurs within individual lower limb muscles during 60 days of bed rest. *J Appl Physiol* 2012;113(10):1545-1559.
29. Johnson MA, Polgar J, Weightman D, Appleton D. Data on the distribution of fibre types in thirty-six human muscles: An autopsy study. *J Neurol Sci* 1973;18(1):111-129.

30. Rutherford OM, Jones DA. Contractile properties and fatiguability of the human adductor pollicis and first dorsal interosseus: a comparison of the effects of two chronic stimulation patterns. *J Neurol Sci* 1988;85(3):319-331.
31. Wiles CM, Jones DA, Young A, Edwards RHT. Relaxation Rate of Type I and Type II Fibres in Human Skeletal Muscle. *Clin Sci* 1978;54(2):31P-32P.
32. Wiles CM, Young A, Jones DA, Edwards RHT. Muscle Relaxation Rate, Fibre-Type Composition and Energy Turnover in Hyper- and Hypo-Thyroid Patients. *Clin Sci* 1979;57(4):375-384.
33. Edwards R, Young A, Hosking G, Jones D. Human skeletal muscle function: description of tests and normal values. *Clin Sci Mol Med* 1977;52:283-290.
34. White MJ, Davies CTM, Brooksby P. The Effects of Short-Term Voluntary Immobilization on the Contractile Properties of the Human Triceps Surae. *Q J Exp Physiol* 1984;69(4):685-691.
35. De Ruiter CJ, De Haan A. Temperature effect on the force/velocity relationship of the fresh and fatigued human adductor pollicis muscle. *Pflügers Archiv* 2000;440(1):163-170.
36. Cartwright MS, Kwayisi G, Griffin LP, Sarwal A, Walker FO, Harris JM, Berry MJ, Chahal PS, Morris PE. Quantitative Neuromuscular Ultrasound in the Intensive Care Unit. *Muscle and Nerve* 2013;47:255-259.
37. Supinski G, Smahaj J, Callahan L. Quadriceps Force Generation in Response to Supramaximal Magnetic Twitch Stimulation of the Femoral Nerve in MICU Patients: Relationship of Muscle Size to Muscle Strength. *Am J Respir Crit Care Med* 2015:A2130.
38. Supinski G, Disselkamp M, Smahaj J, Callahan L. Effect of NMES on Quadriceps Strength in Mechanically Ventilated Patients. *Am J Respir Crit Care Med* 2015:A2678.
39. Ansved T. Effects of immobilization on the rat soleus muscle in relation to age. *Acta Physiol Scand* 1995;154(3):291-302.
40. Diffie G, Caiozzo V, Herrick R, Baldwin K. Contractile and biochemical properties of rat soleus and plantaris after hindlimb suspension. *Am J Physiol* 1991;260(3 Pt 1):C528-534.

41. Winiarski A, Roy R, Alford E, Chiang P, Edgerton V. Mechanical properties of rat skeletal muscle after hind limb suspension. *Exp Neurol* 1987;96(3):650-660.
42. Grimm A, Teschner U, Porzelius C, Ludewig K, Zielske J, Witte OW, et al . Muscle ultrasound for early assessment of critical illness neuromyopathy in severe sepsis. *Critical Care* 2013;17:R227.
43. Sarwal A, Parry SM, Berry MJ, Hsu F-C, Lewis MT, Justus NW, et al Interobserver Reliability of Quantitative Muscle Sonographic Analysis in the Critically Ill Population. *J Ultrasound Med* 2015;34(7):1191-1200.
44. Caresio C, Molinari F, Emanuel G, Minetto MA. Muscle echo intensity: reliability and conditioning factors. *Clin Physiol Funct Imaging* 2015;35(5):393-403.
45. Kleyweg RP, van der Meche FG, Schmitz PI. Interobserver agreement in the assessment of muscle strength and functional abilities in Guillain-Barre syndrome. *Muscle Nerve* 1991;14(11):1103-1109.

FIGURE LEGENDS

Figure 1. Change in ankle dorsiflexor force (100HzAD) between testing occasions (n=13). n=13 patients assessed on each occasion

Figure 2. Individual force-frequency curves for patients assessed on first (n=13) and second (n=12) testing occasion. Left panel, n=13 first testing occasion; Right panel, n=12, second testing occasion

Abbreviations: TwAD – single twitch ankle dorsiflexor muscle force

Figure 3. Force frequency responses of the ankle dorsiflexors with 1 s trains of supramaximal electrical peroneal nerve stimulation at 5-100Hz. Top panel shows an example trace, including single twitch response, and bottom panel the mean (SD) ankle dorsiflexor muscle force for critically ill patients measured at the first testing occasion (filled circles) and second testing occasion (open squares). Points at bars represent values for mean and standard deviation respectively. First testing

occasion, n=13, normalised ADMF (%100HzAD) per stimulation frequency: 5Hz, 30.6±14.1; 10Hz, 46.7±20.1; 20Hz, 68.8±21.0; 30Hz, 82.4±16.1; 40Hz, 85.3±18.9; 50Hz, 91.7±13.8; 80Hz, 93.3±13.7.

Second testing occasion, n=12, normalised ADMF (%100HzAD) per stimulation frequency: 5Hz, 33.4±12.6; 10Hz, 53.3±16.8; 20Hz, 72.1±20.8; 30Hz, 81.7±14.8; 40Hz, 88.2±10.7; 50Hz, 94.7±10.4;

80Hz, 98.7±5.4. *Abbreviations: ADMF – ankle dorsiflexor muscle force*

AUTHOR CONTRIBUTIONS

BC was responsible for study design, data acquisition, analysis and interpretation, and drafted and revised manuscript. MM contributed to data acquisition, analysis and interpretation. VM contributed to data acquisition. WB and PH facilitated data acquisition. NH contributed to data interpretation. GFR was responsible for conception and contributed to study design, data acquisition and interpretation and manuscript revision. All authors contributed to manuscript revision and agreed the final version for submission. BC and GFR act as the guarantors for the intellectual integrity of the work.

TABLES

Table 1. Clinical characteristics of patients

Characteristic	Whole cohort (n=21)	Patients recruited within 24hours ICU admission (n=16)	Patients tested on both occasions (n=13)
Age (years)	66.7±15.9	72.2±12.0	66.2±15.3
Sex (M:F)	20:1	15:1	12:1
Chronic co-morbidity*			
Cardiac	12 (57.1)	10 (62.5)	7 (53.8)
Respiratory	5 (23.8)	4 (25.0)	4 (30.8)
Liver	4 (19.0)	1 (6.3)	1 (7.7)
Renal	2 (9.5)	1 (6.3)	1 (7.7)
Other	5 (23.8)	5 (31.3)	5 (38.5)
APACHE II	23.1±5.7	23.6±6.2	21.9±6.5
SOFA score [#]	12.4±3.2	11.8±2.7	12.0±2.7
MOF [#]	19 (90.5)	16 (100.0)	12 (92.3)
BMI (kg/m ²)	22.1 (21.2-23.1)	22.0 (21.2-23.7)	22.5 (21.4-23.1)
Admission category			
Medical	18 (85.7)	15 (93.8)	10 (76.9)
Surgical	3 (14.3)	1 (6.2)	3 (23.1)
ICU LOS (days)	13.0 (9.0-25.5)	12.0 (8.0-26.8)	12.0 (8.0-46.0)
Hospital LOS (days)~	58.1±51.4	61.6±54.6	57.1±54.0
Survival			
ICU	14 (66.7)	11 (68.8)	9 (69.2)
Hospital	12 (57.1)	9 (56.3)	9 (69.2)
Between testing occasions (n=13)			
MV (days)			5.0±2.7

MOF (days)	7.0 (5.0-7.0)
SOFA score**	9.6±3.4
Pharmacotherapy (number of patients; days)	
Inotropes	11; 4.4±2.4
Vasopressors	4; 0.0 (0.0-1.5)
Sedation	12; 5.0±1.8
Analgesia	12; 4.8±1.9
NMBA	0;
Steroids	0;
Renal replacement therapy	7 (53.8%)
Fluid balance (l)**	5.9±5.8
Blood glucose level (mg/dl)**	126.0±28.8
Insulin (IU)**	111.0 (91.5-262.0)
Creatinine (mg/dl)**	1.4 (1.0-2.0)
Blood urea nitrogen (mg/dl)**	30.0±14.3

Data reported as mean±SD, median (interquartile range) or n (%). *Chronic comorbidities reflect all comorbidities reported in patients, therefore sum exceeds cohort number. [#]At ICU admission. [~]Indicates post-ICU discharge hospitalisation. **SOFA score, blood glucose level, insulin, creatinine and urea are group average values of individual average daily values between testing occasions; fluid balance is the average individual cumulative fluid balance between testing occasions.

Abbreviations: APACHE – acute physiology and chronic health evaluation. SOFA = sequential organ failure assessment. MOF = multi-organ failure. BMI = body mass index. ICU = intensive care unit. LOS = length of stay. MV = mechanical ventilation. CVVHF = continuous venous-venous haemofiltration. NMBA = neuromuscular blocking agents

Table 2. Ankle dorsiflexor muscle force and peripheral skeletal muscle architecture in patients

Parameter	Recruited within 24hours ICU admission (n=16)	Recruited after Day 1 of ICU admission (n=5)
TwAD (kg)	3.3 (2.6-4.3)	1.7 (1.2-10.4)
100HzAD (kg)	9.8 (8.9-16.0)	8.4 (2.4-12.5)
100HzAD _{pred} (kg)	20.67 (16.5-23.6) [#]	23.4 (19.5-27.4)
100HzAD _{%pred} (%)	56.0 (44.1-63.8) [#]	30.5 (10.4-60.1)
TwAD/MRR (kg/s)	0.002 (0.002-0.003)	0.001 (0.0007-0.002)
TA _{CSA} (mm ²)	853.3 (704.3-1043.0)	824.7 (692.2-1082.0)
TA _{EI} (AU)	47.5 (42.0-51.8)	47.0 (29.7-49.0)
TA _{EI(SD)} (AU)	30.6 (27.8-33.4)	23.2 (18.2-35.2)
TA _{PA} (°)	7.5 (6.9-8.1)	6.9 (6.6-7.2)
TA _{Scthickness} (cm)	3.3 (2.6-4.3)	4.6 (3.1-5.4)
TwAD/TA _{CSA} (g/mm ²)	3.9 (2.8-5.1)	1.5 (1.2-17.0)
100HzAD/TA _{CSA} (g/mm ²)	13.4 (10.5-16.6)	11.4 (3.1-57.2)
TwAD:100HzAD	0.3 (0.2-0.4)	0.3 (0.2-0.4)
RF _{CSA} (mm ²)	638.2 (521.0-747.6)	628.8 (373.5-1051.0)
RF _{EI} (AU)	52.0 (43.5-59.4)	55.6 (48.4-59.6)
RF _{EI(SD)} (AU)	29.1 (24.8-34.4)	32.8 (23.4-33.7)
RF _{PA} (°)	7.6 (7.1-8.4)	7.5 (6.1-8.4)
RF _{Scthickness} (cm)	0.8 (0.6-1.0)	1.4 (0.8-1.8)

Data are presented as mean±standard deviation or median (interquartile range). [#]n=14 due to missing 100HzAD data (n=1) and missing TA_{CSA} data (n=1) for inputting into prediction equation. 100HzAD for n=15 patients, 9.8 (8.9-16.0)kg; significantly lower than predicted values (p=0.0001).

Abbreviations: TwAD = twitch ankle dorsiflexor force. 100HzAD = 100Hz ankle dorsiflexor force. MRR = maximum relaxation rate.

TA_{CSA} = tibialis anterior cross-sectional area. TA_{EI(SD)} = tibialis anterior echogenicity (standard deviation). TA_{PA} = tibialis anterior pennation angle. TA_{sc thickness} = tibialis anterior subcutaneous thickness. RF_{CSA} = rectus femoris cross-sectional area. RF_{EI(SD)} = rectus femoris echogenicity (standard deviation). RF_{PA} = rectus femoris pennation angle. RF_{sc thickness} = rectus femoris subcutaneous thickness

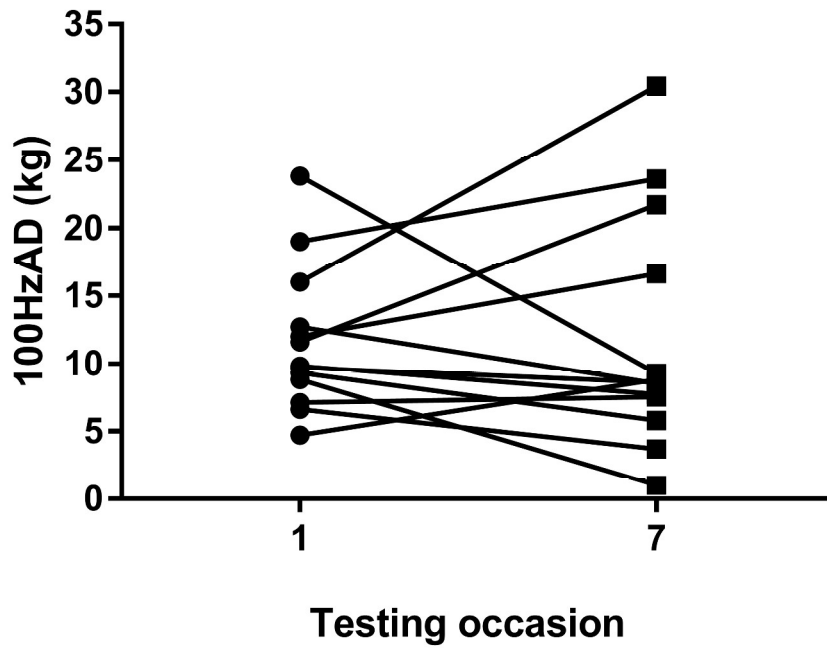
Table 3. Ankle dorsiflexor muscle force and peripheral skeletal muscle architecture on repeat occasions

Parameter	Testing occasion 1	Testing occasion 2	Δ	p
TwAD (kg)	2.9 (2.0-3.6)	3.0 (2.0-4.1)	0.5 (-0.9-1.1)	0.5
100HzAD (kg)	9.8 (8.0-14.4)	8.6 (6.7-19.2)	-1.1 (-3.9-4.6)	0.9
100HzAD _{pred} (kg)	21.8 (17.9-26.6) [#]	19.3 (14.8-24.1) [#]	-2.9 (-4.4- -1.6)	0.01
100HzAD _{%pred} (%)	56.0 (43.2-64.1) [#]	51.2 (34.7-77.7) [#]	12.1 (-17.2-23.9)	0.6
TwAD/MRR (kg/s)	0.002±0.0008	0.002±0.0009	-0.0003±0.0009	0.2
TA _{CSA} (mm ²)	921.8 (752.2-1055.0)	800.8 (678.8-961.1)	-84.3 (-133.4- -54.2)	0.005
TA _{EI} (AU)	47.4 (39.3-51.4)	42.2 (39.1-52.6)	2.3 (-3.6-7.5)	0.5
TA _{EI(SD)} (AU)	29.1 (26.1-32.1)	27.6 (24.4-33.1)	-0.2 (-2.0-4.0)	0.9
TA _{PA} (°)	7.1 (6.8-7.9)	7.1 (6.7-7.7)	-0.1 (-0.6-0.3)	0.7
TA _{Scthickness} (cm)	0.3 (0.3-0.4)	0.3 (0.2-0.5)	-0.02 (-0.06-0.1)	0.6
TwAD/TA _{CSA} (g/mm ²)	3.5 (2.1-4.0)	3.9 (2.3-5.3)	0.8 (-0.5-2.0)	0.2
100HzAD/TA _{CSA} (g/mm ²)	13.2 (10.5-16.4)	11.7 (8.9-20.0)	1.8 (-4.3-5.9)	0.8
TwAD:100HzAD	0.3 (0.2-0.4)	0.3 (0.2-0.4)	0.01 (-0.04-0.2)	0.2
RF _{CSA} (mm ²)*	632.2 (511.4-837.3)	553.8 (394.6-621.1)	-95.75 (-199.9- -48.8)	0.0049
RF _{EI} (AU)*	56.3 (50.6-61.3)	47.5 (41.5-58.2)	-5.9 (-14.8- -1.7)	0.02
RF _{EI(SD)} (AU)*	32.0 (29.1-34.9)	26.3 (23.6-29.9)	-5.3 (-10.5- -0.8)	0.03
RF _{PA} (°)*	7.3 (7.0-8.0)	7.4 (6.3-8.2)	-0.2 (-0.8-0.9)	0.9
RF _{Scthickness} (cm)*	0.89 (0.62-1.21)	0.95 (0.74-1.19)	-0.02 (-0.12-0.29)	1.0

Data are presented as median (interquartile range). n=13. p values derived from Wilcoxon signed rank tests (non-parametric data) and represent differences between testing occasion 1 and 2. *n=12: complete rectus femoris muscle architecture data not acquired in one patient due to obesity and rectus femoris pennation angle not measurable in a further four patients due to poor quality images.. [#] Observed values significantly reduced compared to predicted on both testing occasions; p=0.0002 (Testing occasion 1) and p=0.008 (Testing occasion 2).

Abbreviations: TwAD = twitch ankle dorsiflexor force. 100HzAD = 100Hz ankle dorsiflexor flexor force. MRR = maximum relaxation rate. TA_{CSA} = tibialis anterior cross-sectional area. TA_{EI(SD)} = tibialis anterior echogenicity (standard deviation). TA_{PA} = tibialis anterior pennation angle. TA_{sc thickness} = tibialis anterior subcutaneous thickness. RF_{CSA} = rectus femoris cross-sectional area. RF_{EI(SD)} = rectus femoris echogenicity (standard deviation). RF_{PA} = rectus femoris pennation angle. RF_{sc thickness} = rectus femoris subcutaneous thickness

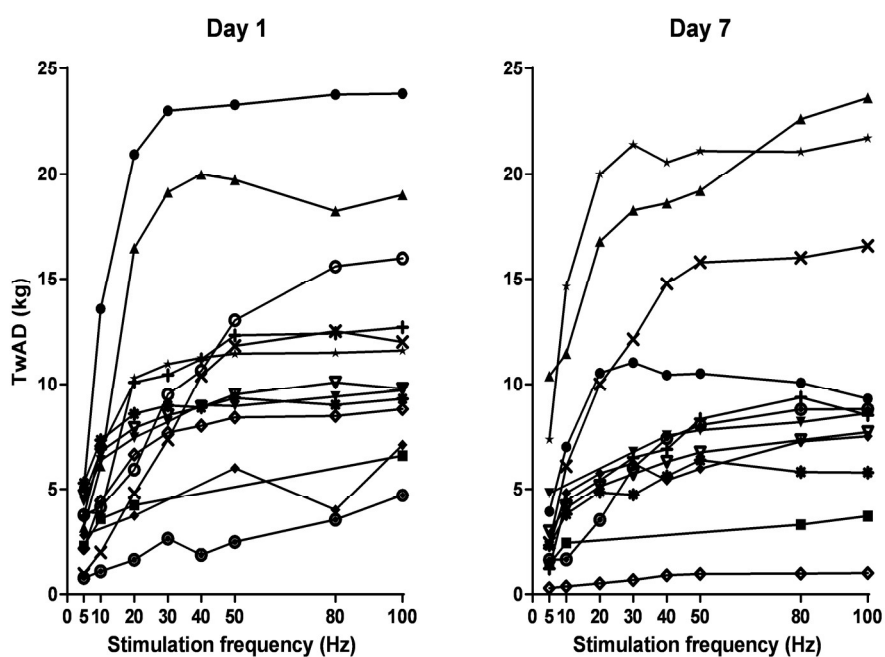
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Change in ankle dorsiflexor force (100HzAD) between testing occasions (n=13)

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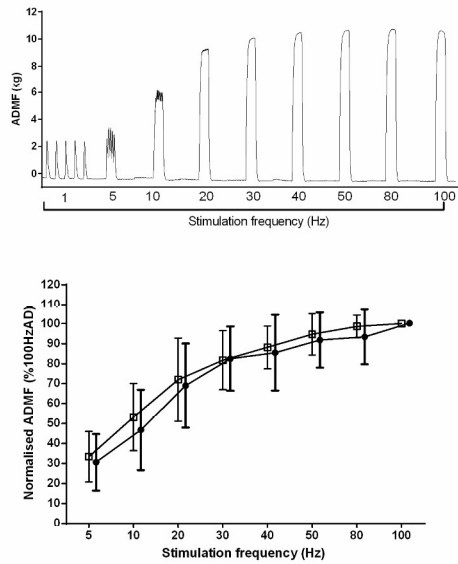
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Individual force-frequency curves for patients assessed on first (n=13) and second (n=12) testing occasion

193x144mm (300 x 300 DPI)

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Force frequency responses of the ankle dorsiflexors with 1 s trains of supramaximal electrical peroneal nerve stimulation at 5-100Hz

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