Early rehabilitation in critical care (eRiCC): Functional electrical stimulation with cycling - protocol for a randomised controlled trial.

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MANUSCRIPT

TITLE: Early rehabilitation in critical care (eRiCC): Functional electrical stimulation with cycling- protocol for a randomised controlled trial.

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ABSTRACT

Introduction: Intensive care acquired weakness is a common problem, leads to significant impairment in physical functioning and muscle strength, and is prevalent in individuals with sepsis. Early rehabilitation has been shown to be safe and feasible; however commencement is often delayed due to a patient's inability to co-operate. An intervention that begins early in an ICU admission without the need for patient volition may be beneficial in attenuating muscle wasting. The eRiCC trial will investigate the effectiveness of functional electrical stimulation-assisted cycling and cycling alone, compared to standard care, in individuals with sepsis.

Methods and Analysis: This is a single centre randomised controlled trial. Participants (n=80) aged ≥ 18 years, with a diagnosis of sepsis or severe sepsis, who are expected to be mechanically ventilated for ≥ 48 hours and remain in the intensive care ≥ 4 days will be randomised within 72 hours of admission to (i) standard care or (ii) intervention where participants will receive functional electrical muscle stimulation-assisted supine cycling on one leg whilst the other leg undergoes cycling alone. Primary outcome measures include: muscle mass (quadriceps ultrasonography; bioelectrical impedance spectroscopy); muscle strength (Medical Research Council Scale; Hand-held dynamometry) and physical function (Physical Function in Intensive care Test; Functional Status Score in intensive care; six-minute walk test). Blinded outcome assessors will assess measures at baseline, weekly, at ICU discharge and acute hospital discharge. Secondary measures will be evaluated in a nested subgroup (n=20) and will consist of biochemical/histological analyses of collected muscle, urine and blood samples at baseline and at ICU discharge.

Ethics and Dissemination: Ethics approval has been obtained from the relevant institution, and results will be published to inform clinical practice in the care of patients with sepsis to optimise rehabilitation and physical function outcomes.

Trial Registration: Australian and New Zealand Clinical Trials Registry ACTRN12612000528853.

ARTICLE SUMMARY

Article Focus:

- Early rehabilitation is now advocated for individuals who are at risk of developing intensive care unit acquired weakness (ICUAW).
- Can FES-assisted cycling or cycling alone minimise muscle mass and strength reductions, and improve discharge physical function, compared with standard care in patients with sepsis?
- What are the cellular and molecular mechanisms responsible for muscle changes in this patient population, and can these be attenuated using FES cycling or cycling alone?

Key Messages:

- This protocol outlines a randomized controlled trial that will investigate the effectiveness of an FES-assisted cycling intervention and cycling alone, commencing within 72 hours of ICU admission compared to standard care
- The results of this trial will provide data to guide the early rehabilitation treatment of patients with sepsis.
- Muscle biopsies and biomarker analyses will provide insights into the effects of sepsis, and an intensive care admission, and early rehabilitation on intracellular signalling pathways and histochemical changes responsible for muscle mass losses

Strengths and Limitations of this study:

- This is the first time that FES-assisted cycling has been investigated within the intensive care unit within 72 hours of admission with sepsis.
- This study combines bench-side research (biopsies and biomarker analyses) with patient centered outcomes (strength and physical function).
- It is a single centre trial so results may need to generalised with caution.

INTRODUCTION

Background:

Intensive care acquired weakness (ICUAW) is a common problem following an ICU admission(1-3) and is associated with prolonged hospitalisation, delayed weaning and increased mortality(4-6). Up to 25% of patients requiring mechanical ventilation (MV) for greater than seven days develop ICUAW(1), and this figure may rise to 50-100% in the septic population(7, 8). Long-term follow-up studies of survivors of critical illness have demonstrated significantly impaired health-related quality of life(9, 10) and physical functioning(11-14) up to five years after ICU discharge, with weakness being the most commonly reported physical limitation(12). Whilst survival has been a main focus of intensive care research, there is a paradigm shift to investigating methods to improve other patient-centred outcomes(15). There has been an increased awareness worldwide of the potential impact and benefit of early rehabilitation in the ICU(15-19). Early rehabilitation in the form of mobilisation has been shown to be safe and feasible(20-24), however it relies on the patient being co-operative, and to have sufficient cardiorespiratory reserve and medical stability(25) to participate in therapy.

Muscle mass is known to reduce by at least 1.6% per day(26), with a 16-20% reduction in muscle mass within the first week in critically ill individuals with severe sepsis(27), indicating that interventions to attenuate muscle wasting in this initial stage may be beneficial. The musculoskeletal system is a highly plastic and adaptive system, responding quickly to changes in the demands placed upon it(3, 28, 29). The pathogenicity and molecular mechanisms for ICUAW have primarily been extrapolated from animal and *in vitro* muscle wasting models(3, 30-33) with ubiquitin-proteasome mediated breakdown postulated to be primarily responsible for the muscle loss observed in critically ill individuals(30, 34-36). Local and systemic inflammatory processes, which occur in critically ill individuals, are thought to lead to a disruption in the balance between muscle protein synthesis and protein breakdown, leading to an overall reduction in muscle mass and force generation capacity(30, 37). Increased circulating inflammatory cytokines (e.g. TNF-α and IL-1ß) may drive mitochondrial oxidative stress and increase intracellular calcium, which are postulated to trigger muscle proteolytic

pathways(30, 38) and may interfere with insulin signalling leading to anabolic resistance,(39) and contribute to electrophysiological inexcitability of the muscle(40). Recent clinical trials in critically ill individuals have demonstrated a reduction in muscle myofibre size with preferential proteolysis of the thick myosin filaments(41, 42), with one trial demonstrating a dramatic increase in protein degradation of up to 160%(42). Currently, the pathogenesis of ICUAW is poorly understood given limited research within human clinical trials(2, 30). Establishing the cellular and molecular mechanisms responsible for loss of muscle mass and strength is essential to help develop future medical and physical therapies.

There is growing interest in the use of assistive technologies to enable patients to commence therapy early in an ICU admission (43). Supine cycle ergometry, which can be utilised passively, actively (by patient effort) or active assisted (using electrical stimulation)(16) has been studied in ICU within one trial with promising results(16). However the intervention did not begin until at least one-week post admission and there were no data reporting frequency of active versus passive cycling(16). Neuromuscular electrical stimulation (NMES) creates passive (i.e. non-volitional) contraction of skeletal muscles through the use of low voltage electrical impulses delivered through to the skin to underlying muscle via surface electrodes(43). It can be commenced early, without the need for patient participation and has been shown to prevent skeletal muscle atrophy in healthy individuals(44) and improve physical function and strength in chronic disease populations, such as heart failure and chronic obstructive pulmonary disease(45). To date, studies within the ICU have involved stimulation of only isolated muscle groups such as the quadriceps, or peroneal muscles, in a resting non-functional position using NMES, with conflicting findings(27, 46-50). Further rigorous research needs to be conducted to determine the optimal stimulation settings, and efficacy of these interventions particularly post ICU on muscle strength and physical function, which is being investigated in one trial currently underway in the United States(51).

Functional electrical stimulation (FES) is different to NMES, as it recruits muscles in functional patterns stimulating them in a similar way to how the muscles would 'normally' contract under

volitional control in healthy individuals. For FES, the majority of the literature to date has been developed within the chronic stroke(52) and spinal cord injury (SCI) populations(53). Alternating recruitment of several muscle groups in a functional activity, such as cycling has been demonstrated in a chronic SCI population to improve the length of time a contraction can be sustained, prior to reaching the point of fatigue(54). This may enable patients to train for a longer period of time, thereby enhancing the training effect. FES-assisted cycling may influence muscle strength and physical function not only at ICU discharge, but also at acute hospital discharge. This trial seeks to examine the combined effect of FES-assisted cycling on muscle mass, strength, and physical function, and compare this with cycling alone, and standard care.

Objectives:

The primary objectives are to:

(1) Evaluate the effectiveness of FES-assisted cycling and cycling alone compared with standard care on muscle mass/cross-sectional area, strength and physical function, in individuals admitted to ICU with sepsis.

(2) Evaluate the effectiveness of FES-assisted cycling compared with cycling alone, on muscle mass, muscle architecture, strength and physical function in individuals admitted to ICU with sepsis.

The secondary objectives are to:

(3) Establish the intracellular signaling pathways and histochemical changes responsible for muscle mass losses in individuals with sepsis;

(4) Inform decisions about outcome measures and power calculations for future studies.

Hypotheses for the primary aims:

(H1) Patients who undergo FES-assisted cycling and cycling alone compared with standard care will have improved muscle mass/cross-sectional area, strength and physical function;

(H2) Patients who undergo FES-assisted cycling when compared with cycling alone will have greater improvement in muscle mass, strength and physical function;

(H3) Patients in the intervention group will have improved activity of anabolic signaling pathways when compared with patients receiving standard care as demonstrated from biomarker analysis.

METHODS:

Trial Design: This is an assessor blinded RCT which will be conducted at Austin Health in Melbourne, Australia.

Participants:

Inclusion and Exclusion Criteria: 80 participants in ICU (Austin Health, Melbourne, Australia) meeting the eligibility criteria will be recruited. *Inclusion Criteria:* (1) ≥ 18 years, (2) expected MV > 48 hours with diagnosis of sepsis or severe sepsis as defined by ACCP Consensus Conference Criteria(55) and (3) predicted ICU LOS ≥ 4 days. E*xclusion Criteria*: (1) known primary systemic neuromuscular disease or intracranial process at admission; (2) lower limb amputation/s; (3) unable to perform study physical outcome measures pre-morbidly due to condition impairing mobility; (4) assessed by medical staff as approaching imminent death or withdrawal of medical treatment within 36 hours; (5) pregnancy; (6) BMI > 40; (7) presence of external fixator or superficial metal in lower limb; (8) open wounds or skin abrasions at electrode application points; (9) presence of pacemaker or implanted defibrillator; (10) transferred from another ICU after > 2 days of consecutive MV; (11) platelets < 40 000 and INR > 1.6 (for biomarker subgroup).

Recruitment Timeframe: This study will take place in a single centre tertiary 20 bed ICU over an 18-month period. The ICU has a throughput of 2,100 patients per year with 200 patients/year who are both septic and requiring MV for > 48 hours.

Recruitment and Randomisation:

Patients meeting the above criteria will be invited to participate. Written informed consent from the patient, next of kin (NOK) or substitute decision maker will be sought. Participants will be asked to provide ongoing consent when able. Participants will be randomly assigned [1:1] to receive either standard care or the intervention using offsite independent randomisation. Concealed allocation will be performed using sequentially numbered opaque sealed envelopes only accessible by research personnel with no involvement in the trial. Intervention arm: leg allocation will be randomly allocated [1:1] to FES-assisted cycling or cycling alone.

This trial involves a two-tier consent process firstly to the FES-assisted cycling intervention and then additionally to biomarker analysis and muscle biopsies with a nested subgroup (n=20) within the primary trial. Details of all participants who refuse consent to muscle biopsy will be recorded. Information regarding screening and flow through this trial will be reported according to the CONSORT (Consolidated Standards of Reporting Trials) Extended Non-Drug guidelines(56). This

trial has been approved by the Human Research Ethics Committee, Austin Health and registered with the Australian and New Zealand Clinical Trials Registry Identifier: ACTRN12612000528853.

Procedure:

The planned flow of participants throughout the study is provided in Figure 1. Once consent is obtained, and prior to randomization; participants will be referred to a physiotherapist who will administer baseline testing of muscle mass/ cross-sectional area (CSA) using diagnostic ultrasound and body composition analysis. Once the patient is awake, a blinded outcome assessor will assess baseline strength and physical function once per week. "Wakefulness" is defined as being able to follow > 3 out of 5 commands as assessed by the De Jonghe's readiness scale(1) with a Riker Sedation Agitation Scale (SAS) of 3-5(57). Biomarker assessment involving muscle biopsies, urine and blood analyses will be assessed at baseline and ICU discharge only. Attrition will be monitored and reasons for withdrawal will be recorded in each trial arm within Figure 1.

Figure 1: Consort diagram giving flow of participants throughout the study

Abbreviations: n, number; MV, mechanical ventilation; ICU, intensive care unit; FES-cycling, functional electrical stimulation-cycling

Standard Care:

Both groups will receive usual medical and nursing care in the ICU and ward settings. In the ICU, physiotherapy will be administered according to a standardized protocol, developed from a previous trial at the same institution. Patients in both arms will undergo usual care physiotherapy including respiratory and mobilisation/rehabilitation (20). Mobilisation activities (including those used for the benefit of the respiratory system) will be provided for up to but not more than 15 minutes. Where possible the level and type of exercise delivered, will be prescribed using the initial PFIT results for each individual patient as per standard care(58). Details of physiotherapy treatment will be recorded but not protocolised on the acute hospital ward.

Intervention:

The intervention will commence within 72 hours of ICU admission, continuing until ICU discharge. Participants will undertake up to one hour of supine cycling daily, ≥ five times weekly using a supine cycle ergometer (RT-300 supine model Restorative Therapies, Ltd., Baltimore, US) attached to a six-channel stimulator (SAGE stimulator, Restorative Therapies Ltd., Baltimore, US) and two RT50 wireless stimulator channels. Surface electrodes will be applied to the gluteal, hamstrings, quadriceps, and calf muscles on both legs. However, the cycling only leg will not have the electrodes switched on (sham).

The intensity of muscle stimulation will be delivered at a level able to cause visible contractions (confirmed by palpation if uncertain) in all muscle groups without causing undue pain or discomfort to the participant. Pain levels will be closely monitored during and post each intervention session (Table 2). If no contractions can be elicited this will be recorded, but the treatment continued, and palpation for contraction assessed at five-minute intervals. FES intensity will be gradually increased to a maximum of 140 mA; with pulse duration of 300 to 400 microseconds (μsecs); frequency between 30 Hz and 50 Hz; and a pedal cadence between 30 to 45 repetitions per minute (rpm). Stimulation parameters will be adjusted to achieve the best possible muscle contraction for the patient. Once the patient is more alert, and able to participate, they will be provided with standardized encouragement to engage in therapy. To increase the intervention workload, resistance (Nm) will be increased incrementally and cycling cadence up to a maximum of 45 rpm. If a participant is readmitted to intensive care, the intervention will be re-instigated until discharge to the acute ward.

Blinding: Whilst participants and intervention physiotherapist cannot be blinded to group allocation, outcome assessors and data analysts will be blinded to group allocation. The success of blinding will be assessed using a short outcome assessor questionnaire for each time point and the number of 'unblinding' episodes will be recorded. If an assessor becomes unblinded, an alternate assessor who is "blinded" will continue to do the measures for that particular individual.

OUTCOME MEASURES

Table 1 provides a summary of the outcome measures and the time-points at which they will be assessed. All primary outcome measures will be measured at baseline; weekly whilst in ICU; at ICU discharge; and acute hospital discharge.

Table 1: Summary of Outcome Measures and time-points of assessment

Abbreviations: MRC: Medical Research Council; HHD: hand-held dynamometry; PFIT: Physical Function in intensive care test; FSS-ICU: Functional Status Score in intensive care; 6-MWT: six-minute walk test.

Baseline descriptive data collection will include age, gender, social, working and smoking history, admission diagnosis, hand/leg dominance, comorbidities as determined by the Functional Comorbidity Index and Charlson Comorbidity Index, severity of illness scoring, and SAS Score. Additionally number of days of sedation and nutritional parameters, will also be recorded. ICU LOS, ICU free days at day 28, mechanical ventilation hours, tracheostomy requirement, incidence of ICU readmission, acute hospital LOS, discharge destination and mortality will also be recorded.

PRIMARY OUTCOME MEASURES:

Muscle Mass:

(1) *Bioelectrical Impedance Spectroscopy (BIS):* Participants will undergo measurement of body composition using multi-frequency BIS machine (ImpediMed SFB7, ImpediMed Ltd., Brisbane, Australia). This will enable calculation of total body water, extracellular and intracellular fluid as well as fat-free mass, and an estimate of muscle mass(59). Measurements will be taken in triplicate with participants in the supine position after single use dual tab gel electrodes have been placed on one foot and one hand on the same side(60). All data will be uploaded onto the BioImped software for subsequent analysis. The reliability of this device in both the critically ill and healthy populations has previously been established (61) and numerous investigators have established the validity of the BIS in detecting within subjects change over time in septic and post surgical populations(62, 63).

(2) Diagnostic two-dimensional (2D) ultrasonography (US) of quadriceps muscle: Rectus Femoris and Vastus Intermedius CSA and thickness will be measured using diagnostic ultrasound (Voluson e BT09 Ultrasound, GE Healthcare, Yokogawa Medical Systems Ltd., Japan). Participants will lie in supine with their leg in passive extension and neutral rotation. A water-soluble transmission gel will be applied to the US head to allow acoustic contact, without depressing the dermal surface. The scanning head will be applied perpendicular to the long axis of the thigh on its anterior surface, approximately three-fifth distance from anterior superior iliac spine to the superior patellar border(64). The point will be marked for consistency of US probe location. Measures will be made in triplicate and averaged. The same operator who is experienced in the measurements will perform all ultrasound examinations. For supplementation of muscle thickness measurements, circumference of the thigh will be assessed using a tape measure at the US measurement site. Reliability and validity of ultrasonography have previously been established both in septic and other medical populations(65-67).

Muscle Strength:

(1) *Medical Research Council Scale (MRC):* manual muscle testing will be scored using the 6-point MRC scale(68).

(2) *Hand-held dynamometry (HHD):* Hand grip and quadriceps strength (supine with 5 inch bolster under knee to enable flexion) (69) will be assessed using HHD bilaterally using a Commander PowerTrack II Dynamometer 1500 (Banner Therapy Ltd., United States) and a Jamar Dynamometer, (Sammons Preston Rolyan, Bolingbrook, United States) respectively. HHD has established reliability and validity(69, 70).

Physical Function:

Will be assessed using several different methods:

(1) *'PFIT' (Physical Function Independence Test)* (71).

(2) *'FSS-ICU' (Functional Status Score in ICU)* (72)

(3) The *'six minute walk test' (6-MWT)* is a commonly used test, which measures the distance that a patient can walk quickly in six minutes over a 25 m flat hallway. It is both self-paced and sub-maximal. The 6MWT has previously been used in the ICU population. This test will only be performed at acute hospital discharge (73)

SECONDARY OUTCOME MEASURES:

Biomarker Analysis: Participants who consent to participation will also be approached for consent to biomarker assessment involving muscle biopsies, blood, and urine samples. Within the intervention arm as previously described, one leg undergoes cycling alone and the other FES-assisted cycling. Therefore muscle biopsies will be collected from both lower limbs in the intervention arm, and in one lower limb in the standard care arm, to allow comparison across the groups. Biomarker assessment will be performed at baseline, and at ICU discharge (where possible the "ICU discharge" measure will occur 24 hours post final intervention exercise session).

Muscle Biopsy:

Using an aseptic technique, under local anesthetic injected into the skin and fascia, muscle biopsies (100-200mg of tissue) will be obtained from the vastus lateralis muscle, 10 cm above the patella. Prior to biopsies being performed, senior medical staff will review anticoagulation profiles, and using the Bergstrom percutaneous needle technique under suction(74) will perform biopsies. Once the tissue has been extracted, part of the biopsy sample (20-50 mg) will be dissected carefully, freed from any visible non-muscle material, embedded in Optimal Cutting Temperature (OCT) compound and immediately frozen in liquid nitrogen-cooled isopentane and stored at -80°C until histochemical analyses. The remainder of the biopsy (100-150 mg) will be immediately frozen in liquid nitrogen and stored at -80°C, until further analyses. Histological assessments of muscle structure and infiltration of inflammatory cells, will be performed using Haematoxylin and Eosin staining, and examination of muscle fibre type specific changes in cross sectional area, oxidative capacity, substrate stores, satellite cell activation, and key proteins regulating protein synthesis and breakdown (mTOR, AMPK, MURF-1 and MAFbx)(75). mRNA and protein expression levels and phosphorylation status of key signaling proteins in the regulation of inflammation will be determined using RT-PCR and standard immune-blotting techniques respectively(76).

Urine analysis:

24-hour urine samples will be collected for analysis of urinary creatinine, 3-Methylhistidine (3-MH) and urea levels. The samples will be stored at -80°C until analysis. 3-MH will be analysed using high performance liquid chromatography (HPLC).

Blood analysis:

Venous blood (15ml) will be collected at each time-point and processed to obtain plasma and serum for subsequent analyses. Stored plasma and serum will be analysed using enzyme linked immunosorbent assay (ELISA) methods for IGF-1, IL-1β, IL-6, IL-10, IL-18 and TNF-α. Blood levels of C-reactive protein (CRP), urea, and creatinine will be retrieved from the standard intensive care nursing charts over a 24-hour period. HPLC will be performed to analyse Vitamin D Levels, serum amino acid concentration levels, and 3-MH levels.

SAFETY PROTOCOLS:

All physiotherapists providing intervention and outcome measurements are experienced ICU clinicians trained in safety and basic life support. Written manuals of protocols and safety guidelines will be made available to all research personnel. Criteria for commencing and ceasing exercise or outcome measurement are outlined in Table 2.

Table 2: Safety Guidelines for Exercise

Abbreviations: mmHg: millimeter of mercury; µg: micrograms; FiO_2: Fraction of inspired oxygen; PEEP: Positive end expiratory pressure; spO_2: saturation of peripheral oxygen; NRS: Numerical Rating Scale.

If any adverse event occurs either during, or up to an hour following intervention or outcome measurement, the chief investigators will be notified. Serious adverse events are defined in this trial as 'adverse events', which results in death, cardiac arrest or stroke. These events will be reported to the Austin Health HREC. A data monitoring committee will examine trial safety by reviewing any adverse events at regular intervals. Research personnel to identify potential safety, recruitment, and treatment issues will review data process indicators based on the consort diagram monthly.

STATISTICS:

Sample Size Calculation: The sample size calculation is informed by data from Burtin et al (2010) using the HHD improvement± SD for quadriceps strength of 0.54±0.76 N/kg. Using alpha = 0.05 and power = 0.80 the total required sample size is 64 cases. Allowing for 14% in hospital mortality and dropouts the sample size has been increased to 80 cases. For biomarker analyses (primarily muscle biopsy) the sample size calculation is based on prior trials detecting changes in muscle protein synthesis and associated signaling following exercise, nutritional and pharmacological interventions

(increase in signaling > 20 % with standard deviation in signaling of 2%)(77, 78). Using alpha =0.05 and power = 0.80 the required sample size is 18 cases. To account for dropouts and mortality we have increased this sample size to 20 cases.

Statistical Analysis: All data will be entered into a purposefully designed database and exported to IBM SPSS Statistical software package (SPSS Inc., Version 20, Chicago, IL) for analyses. Descriptive statistics will first be used to examine the distribution for the two groups comparing baseline scores, to describe mean outcomes, and test whether parametric test assumptions have been met. Analyses will be by intention to treat. Statistical significance will be set at $p < 0.05$ (two-tailed). Possible differential attrition will be assessed by comparing baseline characteristics of those who withdraw or die against those who remain in the trial. Linear mixed modeling will be used to assess the treatment effects. Imputation of missing data will not be undertaken. Adjustment will be undertaken if significant imbalance is evident in baseline covariates and in this case results of both the adjusted and unadjusted results will be described(79). As a secondary analysis mean change in scores, 95% confidence intervals, and comparison with minimal clinically important differences in outcomes will be presented.

ETHICS AND DISSEMINATION:

The Austin Health HREC has granted ethical approval for this trial. Trial results will be disseminated widely, through peer review journal manuscripts and scientific conference presentations. The investigators will submit trial progress summaries to all sponsors of the trial on a regular basis.

RESULTS:

The trial will determine whether FES-assisted cycling, or cycling alone compared to standard care will improve muscle mass, strength and physical function. The cellular and molecular mechanisms, responsible for the observed muscle mass and strength changes will be examined using biomarker analyses.

DISCUSSION:

ICU-AW is a well-recognized clinical problem especially in individuals with sepsis. The effects of this syndrome have been shown to last for many years, with physical function one of the most affected patient outcomes. This research is innovative in that it combines both clinical and basic sciences to evaluate the mechanisms of change together with measuring patient centred outcomes. It will be the first interventional trial to evaluate the effectiveness of FES-assisted cycling and cycling alone on muscle mass, architecture, strength and physical function. The important primary outcomes for the patient are expected to be improved or maintained strength, and physical function. Given the potentially devastating effects of a critical illness on the survivors and their families(15), gaining

improved strength and physical function will be critical outcomes for all ICU survivors. The study design will allow future decisions to be made about sample sizes and primary outcomes to inform future research. The evaluation of an early rehabilitation programme involving FES-assisted cycling, which can commence in the critical period where the majority of muscle reductions occur, may provide direction for the development of appropriate rehabilitation in survivors of ICU.

LIST OF ABBREVIATIONS:

ACCP: American College of Chest Physicians; **AMPK:** AMP-activated protein kinase; **ANOVA:** analysis of variance; **ANZCTR:** Australian and New Zealand Clinical Trials Registry; **APACHE II:** Acute Physiology and Chronic Health Evaluation II; **BIS:** Bioelectrical impedance spectroscopy; **BMI:** body mass index; **CONSORT:** Consolidated Standards of Reporting Trials; **CRP:** C-reactive protein; **CSA:** cross sectional area; **ELISA:** Enzyme linked immunosorbent assay; **FES:** functional electrical stimulation; **FiO_2:** Fraction of inspired oxygen; **FSS-ICU:** Functional status score in intensive care; **HHD:** hand-held dynamometry; **HPLC:** high performance liquid chromatography; **HREC:** Human Research Ethics Committee; **Hz:** Hertz; **IL-6:** Interleukin-6; **IL-10:** Interleukin-10; **IL-18:** Interleukin-18; **IL1ß**: Interleukin-1 beta; **ICU:** intensive care unit; **ICUAW:** intensive care-acquired weakness; **IGF-1:** Immunoglobulin Factor-1; **INR: i**nternationalized normal ratio; **LOS:** length of stay; **mA:** milliamplitude; **MAFbx:** muscle atrophy F-box; **mmHg:** millimeter of mercury; **MRC:** Medical Research Council Scale; **mRNA:** messenger ribonucleic acid; **mTOR:** mammalian target of rapamycin; **MURF-1:** muscle ring-finger protein-1; **MV:** Mechanical ventilation; **N/Nm:** Newton metre (Force); **NOK:** next of kin; **NMES:** Neuromuscular electrical stimulation; **NRS:** Numerical Rating Scale; **OCT:** optimal cutting temperature compound; **PEEP:** positive end expiratory pressure; **PFIT:** Physical Function independence test; **RCT:** randomized controlled trial; **rpm:** repetitions per minute (cadence); **RT-PCR:** real-time polymerase chain reaction; **SAS:** Riker Sedation-Agitation Scale; **SCI:** spinal cord injury; **SD:** standard deviation; **spO_2**: saturation of peripheral oxygen; **SPPB:** Short Performance Physical Battery; **TNF-α:** Tumour Necrosis Factor Alpha; **US:** ultrasound/ultrasonography; **µg**: micrograms; **µsecs:** microseconds; **2D:** Two-dimensional; **3-MH:** 3-Methylhistidine; **6-MWT:** six-minute walk test

COMPETING INTERESTS: None to declare

AUTHORS' CONTRIBUTIONS:

All authors contributed to the study design, method and writing. SP, LD, SB, RK, NH, AB, SW helped to develop the successful grant proposals, which support the study. SW, and NH were involved in providing advice on safety; monitoring and ethical issues and LD will chair the data monitoring committee. SP, AB and DE contributed to the ultrasound protocol and analysis component of the trial. RK, ZP, NH and SW have specifically contributed to the biomarker analysis component of the trial.

SP drafted the manuscript. All other authors critically revised it for important intellectual content. All authors contributed to and approved the final version of the manuscript for publication.

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Table 1: Summary of Outcome Measures and time-points of assessment

	Baseline	Weekly in ICU	ICU discharge	Acute Hospital Discharge
Muscle composition and thickness measures:				
Bioimpedance spectroscopy	✓	✓	✓	✓
Quadriceps Ultrasonography	✓	✓	✓	✓
Strength Measures:				
MRC score	✓	✓	✓	
HHD grip and quadriceps	✓	✓	✓	✓
Physical Function Measures:				
PFIT	✓	✓	✓	
FSS-ICU score	✓	✓	✓	
6-MWT				✓
Biomarker Measures:				
Muscle biopsy	✓		✓	
Urine analyses	✓	✓	✓	
Blood analyses	✓	✓	✓	

Abbreviations: MRC: Medical Research Council; HHD: hand-held dynamometry; PFIT: Physical Function in intensive care test; FSS-ICU: Functional Status Score in intensive care; 6-MWT: six-minute walk test.

Table 2: Safety Guidelines for Exercise:

Safety Guidelines: Exercise should not be delivered or should be ceased when:
1. Patient mean arterial blood pressure < 65 or > 120 mmHg or ≤ 10 mmHg lower than normal systolic or diastolic in renal patients
2. Patient heart rate is < 50 or > 140 beats/minute or new arrhythmia develops (including ventricular ectopics or new onset atrial fibrillation)
3. Patient requires> 30 µg of noradrenaline or comparable inotropic or vasopressor support
4. Patient complains of new onset chest pain
5. Patient becomes pale or sweaty and/or patient specifically requests to stop due to feeling acutely unwell
6. Presence of extracorporeal membrane oxygenation or intra-aortic balloon pump
7. FiO_2 > 0.8
8. PEEP > 15 cmH_20
9. Respiratory rate > 35 breaths/minute sustained for > 60 seconds
10. spO_2 falls > 10% below resting level or < 85% for > 60 seconds
11. Numerical rating scale (NRS) pain level remains > 7 for five minutes despite adjusting stimulation intensity. Pain levels will be monitored using NRS if patient is awake at commencement and five minutes into the exercise session. If the patient is intubated we will use standardized assessment of pain including facial grimacing, ventilator dysinchrony, and agitation levels to monitor pain levels during exercise

Abbreviations: mmHg: millimeter of mercury; µg: micrograms; FiO_2: Fraction of inspired oxygen; PEEP: Positive end expiratory pressure; spO_2: saturation of peripheral oxygen; NRS: Numerical Rating Scale.

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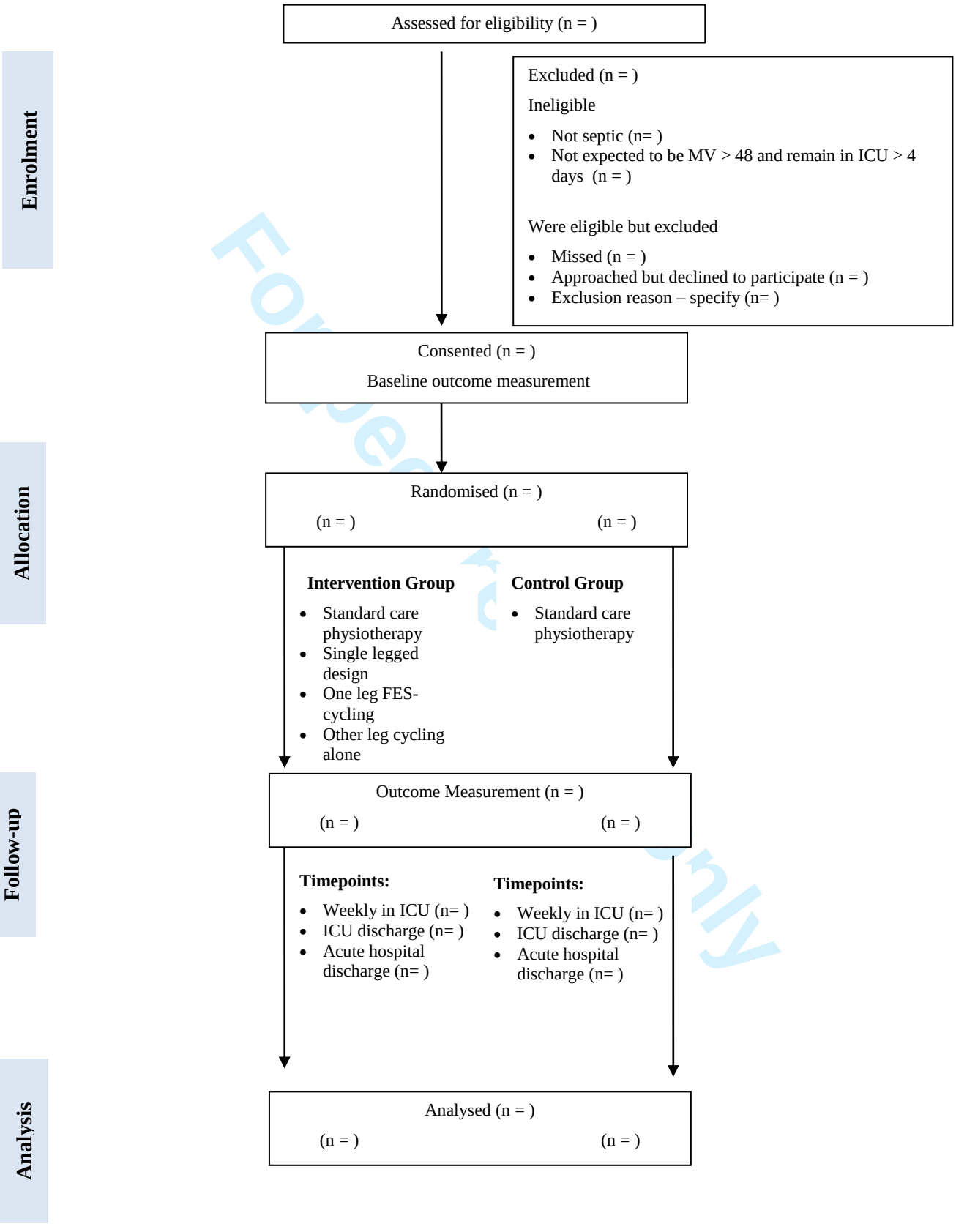
Figure 1: Consort diagram giving flow of participants throughout the study



Abbreviations: n, number; MV, mechanical ventilation; ICU, intensive care unit; FES-cycling, functional electrical stimulation-cycling.