# Study Protocol (2021-12-08)

## Investigating the Anabolic Response To resISTance exercise during critical illness: The ARTIST-1 Randomized Controlled Trial.

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## Background

The debilitating impact of critical illness has been recognized for several decades. Disability related to intensive care is now described as a syndrome called ICU-acquired weakness (ICUAW). ICUAW affects up to 70% of ICU patients and is most common with higher illness severity. The pathophysiology is heterogenous, and involves muscle protein breakdown, altered bioenergetics, axonal injury and impaired excitation-contraction coupling. Patients that develop ICUAW require longer hospitalization and have a higher risk of death [1].

Weakness also has significant long-term consequences. In a landmark study, Herridge et al observed that predominantly young and previously healthy survivors of acute respiratory failure had reduced exercise capacity up to five years after ICU stay [2]. Physical function was unrelated to residual pulmonary dysfunction. Long-term disability was associated with significant health care costs, delayed return to work, and overall poor quality of life.
Preventing muscle atrophy is a potential way to counteract weakness. Critical illness is associated with a rapid loss of skeletal muscle, which may exceed 10% of lean body mass in the first week of intensive care [3,4]. Dos Santos et al found that a majority of ICU survivors had not recovered their muscle volume up to six months after hospital discharge [5].
During critical illness there is a dramatic increase in muscle protein breakdown, while synthesis is unaltered [6]. The result is a net loss of total body protein. Aging and comorbid conditions also contribute toward a resistance to anabolic signals, which may be exacerbated by immobilization and systemic inflammation [7]. Our research group has previously found that nutritional interventions such as amino acid supplementation can improve whole-body protein balance in ICU patients [8,9], but the effect on muscle metabolism is still unknown. Studies in exercise physiology have demonstrated that resistance training and amino acid ingestion have synergistic effects on muscle protein synthesis in healthy subjects [10]. It is therefore an

appealing therapy to counteract muscle wasting in ICU, and to regain muscle mass during convalescence.

Despite several clinical trials, there remains equipoise regarding the efficacy of exercise in improving physical function in-ICU or after discharge [11,12]. These mixed signals are unsurprising given the heterogeneous causes of ICUAW. Only a few studies in this field assess muscle architecture or cellular signaling in response to training [13,14]. However, the gold standard in determining the anabolic response to exercise is to directly measure the effect on protein synthesis and breakdown. To our knowledge there is still no published research using this methodology in critically ill patients.

# Aim and hypothesis

The overall aim of this project is to determine the anabolic response to resistance exercise during and after critical illness.

The investigators hypothesize that resistance exercise, in addition to amino acid supplementation and routine physiotherapy, results in an improved muscle protein balance in ICU patients compared to amino acid supplementation and routine physiotherapy alone (primary outcome). The effect of the intervention on other parameters of muscle protein kinetics and within-group differences in protein kinetics before and after physiotherapy will be assessed as secondary outcome measures.

# Methods

## Study design

Single-center randomized controlled assessor-blind clinical trial.

## Population

### Inclusion criteria

1. Adult (≥18 years) patient admitted to the ICU of the study site.
2. Patient deemed suitable for active mobilization by the attending physician and physiotherapist.

3. Not expected to be discharged or transferred from the unit within 24 h.
4. Functioning arterial catheter in situ.

### Exclusion criteria

1. Not able to provide informed consent.
2. Systemic anticoagulation with LMWH/UFH/DOAC in therapeutic dose range for deep vein thrombosis or pulmonary embolism, or dual antiplatelet therapy. If LMWH is administered twice daily, the patient is eligible for participation provided that vascular access is performed at nadir prior to the first daily dose.
3. Clinically significant inherited or acquired disorder of hemostasis.
4. Morbid obesity that interferes with femoral cannulation or doppler measurements.
5. Hemodynamic instability requiring ongoing volume resuscitation with crystalloid solutions or blood products.
6. Lower-limb amputee.
7. Lower-limb artheriosclerotic disease with critical ischemia.
8. Metastatic cancer or active hematological malignancy.
9. Inherited disorder of amino acid metabolism.
10. Chronic muscle, neuromuscular och neurologic disease with prior documentation of clinically significant lower-limb involvement.
11. Pregnancy.
12. CAM-ICU screening positive for delirium.
13. Single organ failure not requiring invasive mechanical ventilation prior to enrollment.

## Intervention and comparator

All research subjects enrolled in the study will perform a physiotherapist-led session of standardized active mobilization, while receiving an intravenous infusion of mixed amino acids (active comparator). Patients randomized to the intervention group will in addition perform a resisted knee extension exercise as a part of their physiotherapy session.

## Outcomes

### Primary outcome

The difference between the experimental and active comparator group in change in lower limb protein balance (nmol Phenylalanine/min) from baseline to post-physiotherapy.

### Secondary outcomes

1. The difference between the experimental and active comparator group in change in lower limb protein synthesis and breakdown (nmol Phenylalanine/min) from baseline to post-physiotherapy.
2. The difference between the experimental and active comparator group in change in lower limb 3-methylhistidine rate of appearance (nmol/min) from baseline to post-physiotherapy.
3. The change in lower limb protein balance, synthesis and breakdown (nmol Phenylalanine/min) in the experimental group, from baseline to post-physiotherapy.
4. The change in lower limb lower limb 3-methylhistidine rate of appearance (nmol/min) in the experimental group, from baseline to post-physiotherapy.
5. The change in lower limb protein balance, synthesis and breakdown (nmol Phenylalanine/min) in the active comparator group, from baseline to post-physiotherapy.
6. The change in lower limb lower limb 3-methylhistidine rate of appearance (nmol/min) in the active comparator group, from baseline to post-physiotherapy.

## Investigation protocol

At the beginning of the protocol, a primed intravenous infusion of labeled ring-$^{2}H_{5}$-phenylalanine (bolus 2.94 µmol/kg, infusion rate 2.94 µmol/kg/h) and $^{2}H_{3}$-methylhistidine (bolus 0.06 µmol/kg, infusion rate 0.06 µmol/kg/h) is started to achieve a steady state of tracer dilution in arterial blood and continued until the end of the protocol. A femoral venous catheter is then sited under local anesthesia for lower limb blood sampling. The physician placing the catheter is free to determine the side of placement depending on the vascular anatomy on ultrasound investigation. A standardized measurement of upper and lower limb muscle thickness is also performed to quantify the baseline muscle mass of study subjects [15].

After 165 minutes of tracer infusion, four 2 ml samples from arterial and lower limb venous blood and femoral artery blood flow measurements on the side of the catheterized leg are performed at five minute intervals. Doppler blood flow measurements will be performed using a high-frequency linear probe with a fixed probe angulation at a level cranial to the branching of the deep femoral artery. The vessel diameter is determined at the same level. Immediately after the baseline measurements, an intravenous infusion of mixed amino acids (Glavamin, Fresenius Kabi) is administered at a rate of 0.1 g/kg/h.

The study subjects will then participate in a physiotherapy session of protocolized active mobilization. While sitting on the side of the bed, patients in the intervention group will be encouraged to perform knee extensions with the leg catheterized for blood sampling, targeting 8-12 repetitions to failure in three sets. Resistance is adjusted using ankle weights. The physiotherapist in charge will document the number of repetitions and other items of the mobilization protocol performed.

After returning to bed rest, new blood samples and blood flow measurements as described above are performed to determine the change in lower limb protein balance every 30 minutes up to 90 minutes post-intervention. At the completion of the protocol the infusions of labeled and unlabeled amino acids are stopped and the femoral vein catheter is removed. The protocol is illustrated in Figure 1.

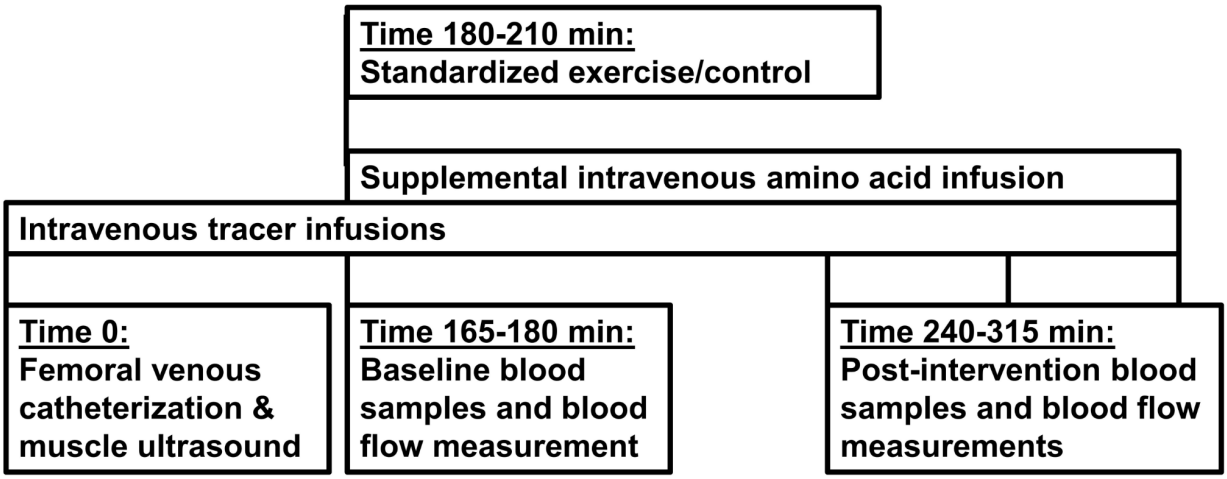


Figure 1. A schematic illustration of the investigation protocol (not to scale).

All other aspects of treatment will be determined by the attending physician and care team, with a request that the rate of enteral and parenteral nutrition remains unchanged from 06:00 in the morning until the end of the protocol.

## Calculations

Lower limb protein balance will be determined using a two-pool model [6]. Net balance (NB) of phenylalanine is derived using the Fick principle:

$NB = (C_A - C_V)*PF$

where $C_A$ = arterial content (nmol/ml); $C_V$ = venous content (nmol/ml); and PF = plasma flow (ml/min).

The rate of appearance (Ra) approximates protein breakdown and is calculated as:

$Ra = C_V*[1-(E_V/E_A)]*PF$

Where $E_A$ and $E_V$ are the arterial and venous enrichments of tracer expressed as molar percentage excess and calculated as an average of the four blood samples in each measurement series.

The rate of disappearance (Rd) approximates protein synthesis and is calculated as:

$Rd = NB + Ra$

## Statistical considerations

### Randomization

Randomization will be performed using a computer-generated sequence in permuted blocks of four. Treatment allocation is concealed in opaque envelopes prepared by a research associate not involved in the study. The envelope is opened by the physiotherapist supervising the exercise session just before the intervention. Research staff in charge of outcome assessment are blinded to allocation until all data analysis is complete.

### Sample size

As variation in muscle protein balance between ICU patients is very large [16], only paired comparisons will allow for studies of small-moderate size to find an effect. Unfortunately, there is no published data that describes the variation in repeated measures of muscle protein balance in ICU patients to support a formal power calculation. The best available estimate of effect size comes from pooled results on changes in whole-body protein balance during nutritional interventions. Using this data, we determine that 10 patients in each treatment arm are required to observe a mean change from negative to neutral lower limb muscle protein balance. To account for potential dropouts we aim to enroll a total of 24 patients.

### Statistics

Between-group differences of continuous outcomes will be analyzed using two-way repeated measures ANOVA. Within-group differences will be analyzed using one-way repeated measures ANOVA. A normal distribution of within-subject change in protein kinetics is assumed from previous observations. The predetermined level of significance is $p \leq 0.05$. Correction for multiple comparisons will not be applied and p-values for secondary outcomes should be considered as exploratory.

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