CLINICAL STUDY PROTOCOL

〔The effects of high protein supplementation, core muscle rehabilitation and neuromuscular electrostimulation (NMES) programs on clinical outcomes in patients with prolonged mechanical ventilation (PMV)〕

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1. Synopsis

Protocol Title： The effects of high protein supplementation, core muscle rehabilitation and neuromuscular electrostimulation (NMES) on clinical outcomes in patients with prolonged mechanical ventilation (PMV)

Study Objectives：

To evaluate the benefit of high protein supplementation and core muscle rehabilitation and trunk NMES intervention for weaning in PMV patients.

Investigational product(s)：

high protein supplementation (1.5 g/kg/day protein)
core muscle rehabilitation (sitting for 30 minutes, twice per day, 5 days per week)
trunk NMES intervention (Electrodes were placed on back designed to activate latissimus dorsi and abdominal wall designed to activate the transversus abdominis and internal and external oblique muscles.
Electrical muscle stimulation was performed by using a using a commercial stimulator (GEMORE, GM300E, Taipei, Taiwan)
with biphasic waves at a simulation frequency of 30 Hz and pulse width of 400s, cycling 2s on and 4s off. Electrical muscle stimulation intensity was gradually increased until a visible muscle contraction was observed (median 60 mA [range 50–65 mA].)

Development Phase： □ I □II □III □IV □其它_____ ■不適用

Study Design：

1. ■ Experimental Group：
    1. High protein supplementation
    2. High protein supplementation + Core muscle rehabilitation
    3. High protein supplementation + Core muscle rehabilitation + trunk NMES intervention

■ Control Group ：

Usual care in Respiratory Care Center

2. Blinding：■ Open ☐ Evaluator-blind ☐ Single-blind(patient) ☐ Double-blind(patient+PI)
☐ Double Dummy ☐ Other _____

3. Randomization: ■ Yes ☐ No

4. ☐ Parallel design ☐ Crossover design ☐ Other _____ ■ Not applicable

5. Treatment Period：3 weeks ☐ Not applicable

6. Study Period: 2 years

6. Dose adjustment：☐ Mandatory ☐ Selectively ☐ No ■ Not applicable

7. Study location：■ Single ☐ Multi-center ☐ Global

Endpoints (Outcome measure)：

1. Primary endpoint:
   Weaning rate (weaning success defined as weaning from ventilator for 5 consecutive days)
2. Secondary endpoints:
   In hospital mortality, Length of mechanical ventilator usage,
   Length of ICU stay and total length of hospitalization,
   Serum albumin level improvement, Creatinine clearance rate

Inclusion/Exclusion Criteria：

Patients

Patients (age ≥ 20 years old) on mechanical ventilator for more than 21 days were recruited from the respiratory care center (RCC). of Chang Gung Memorial Hospital in Taiwan.

The **inclusion criteria** were as follows:

1. age ≥ 20 years old
2. using mechanical ventilator for more than 21 days (including patients under tracheostomy or endotracheal tube)
3. stable clinical condition, without using inotropic agent
   (arterial blood gas pH 7.35–7.45, PaO_2≥60 mm Hg at FiO_2 40%, absence of signs and symptoms of uncontrolled infection, and hemodynamic stability)
4. maximal inspiratory pressure (MIP) < 30mmHg
5. under enteral nutrition (EN) via NG tube.

The **exclusion criteria** were as follows:

1. Acute infection and sepsis (fever up to 38.5 degree)
2. Severe neuromuscular disease, or uncontrolled epilepsy
3. Bony fracture or DVT history
4. Wound over the abdomen
5. Congestive heart failure with EF < 40% or using pacemaker
6. BMI>35 kg/M^2, or severe edema
7. Patients with hepatic failure, rapid progressed malignancy, or pregnancy were also excluded.
8. Under parenteral nutrition (PN)
9. Use pacemaker

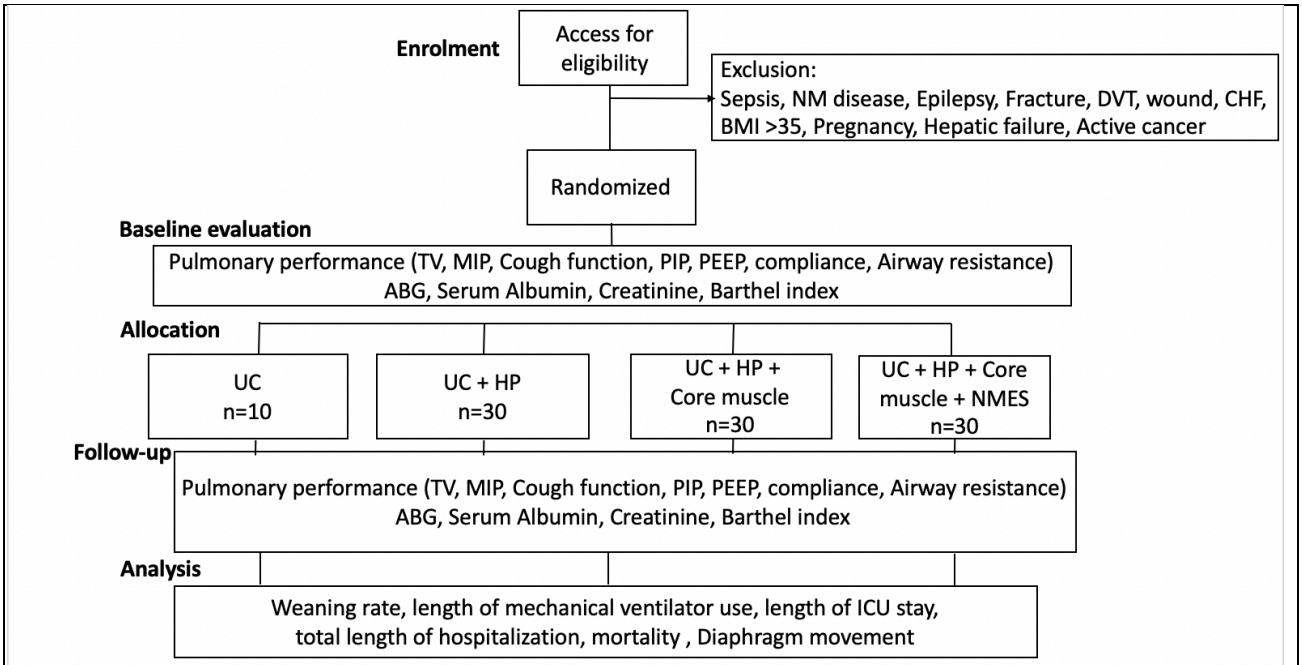
Patient's identification will be recorded as a study code, which will not be mentioned in the publication.

Study Procedures：

Eligible patients with informed consents will randomly stratify into four groups: (1) Usual care (UC), (2) UC + high protein diet (HP), (3) UC + HP + core muscle rehabilitation, (4) UC + HP + core muscle rehabilitation + neuromuscular electric stimulation (NMES). Ten patients will be assigned to UC group. Thirty patients will be included in another 3 group. An independent researcher from Chang Gung Medical Foundation Clinical Trial Center makes random allocation cards using computer-generated random numbers. We used sequential sealed envelopes were prepared by an independent investigator (Chung-Chi Huang), with one chosen randomly by study nurse for each subject. The subjects were then assigned to each group according to the label in the envelope. The total daily caloric intake is based on the suggestion of nutritionist. The HP groups will maintain unchanged total daily caloric intake and increasing protein content to 1.5g/kg/day. The specific formulas were chosen for their different energy and protein requirement (mainly Nutri-Aid, or NU-PEP HN, according to patients' digestion, and may add Whey-Aid if necessary (above formula are available in CGMH currently)). We will maintain the nutrition strategies for 21 days according to patients' weaning condition. Protein provision was not reduced in case of renal failure. Core muscle rehabilitation is sitting on bedside with or without aids, for 30 minutes, twice per day, 5 days per week, for 3 weeks. NMES was applied for 30 min, twice per day, 5 days per week, for 3 weeks via surface rectangular electrodes. Electrodes were placed on back designed to activate latissimus dorsi and abdominal wall designed to activate the transversus abdominis and internal and

external oblique muscles as previously McCaughey et al described [37]. Electrical muscle stimulation was performed by using a commercial stimulator (GEMORE, GM300E, Taipei, Taiwan) with biphasic waves at a simulation frequency of 30 Hz and pulse width of 400s, cycling 2s on and 4s off. Electrical muscle stimulation intensity was gradually increased until a visible muscle contraction was observed (median 60 mA [range 50–65 mA].

The age, gender, smoking history, diagnosis, length of mechanical ventilator use, APACHE II will be recorded. The pulmonary performance, including tidal volume, maximal inspiratory pressure (MIP), cough function, peak inspiratory pressure (PIP), positive end-expiratory pressure (PEEP), lung compliance, airway resistance, blood gas analysis, Barthel index, serum albumin, creatinine, electrolytes will record before, during (twice per week) and after intervention. We also follow the outcome of hospitalization, including the weaning rate, mortality, length of mechanical ventilator use, length of ICU stay and total length of hospitalization. We will further evaluate the diaphragm movement. Ultrasound was performed at the end of exhalation (without stimulation) to measure the thickness of the diaphragm, twice per week of participation, and then weekly until RCC discharge. All measurements were taken from muscles on the right-hand side of the participant by the same assessor at all assessment sessions. For the diaphragm, the probe was placed parallel to the anterior axillary line in the intercostal space between the 9th and 10th rib and moved in the cranial and caudal directions until the pleural line was identified. From this point, the probe was moved approximately 1 or 2 intercostal spaces lower to identify the costal diaphragm in the zone of apposition. In all measurements, the probe was placed perpendicular to the skin. We will analyze the diaphragm movement, thickness and the correlation with successful weaning rate.



Abbreviation: RCC, respiratory care center; PMV, prolonged mechanical ventilation; NM, neuromuscular; DVT, deep vein thrombosis; CHF, congestive heart failure; BMI, body mass index; TV, tidal volume; MIP, maximal inspiratory pressure; PIP, peak inspiratory pressure; PEEP, positive end-expiratory pressure; UC, usual care; HP, high protein, NMES, neuromuscular electrical stimulation

Concomitant Treatments： ■不適用

1. Concomitant Therapy：

2. Prohibited Therapy：

Statistical Methods：

1. Main study Hypothesis：☐ Equality ■ Superiority ☐ Non-inferiority ☐ Equivalence ☐ Other _____

2. Estimated Sample Size：100_

3. Efficacy assessment group：■ Intent-to-treat (ITT) ☐ Per-Protocol (**PP)** ☐ Other _____

ITT: We include all eligible patients who meet the criteria had signed informed consents to participate this study.

4. Interim analysis：☐ Yes ■ No

Statistical methods：Categorical variables were described using counts (percentages). Parametric data were expressed as means ± standard deviation (SD). Among the group

comparisons, one-way analysis of variance (ANOVA) followed by Dunnett's test, where appropriate, was used to determine the statistical significance of the difference between means. The follow-up parametric data were compared using the paired student's t-test. Non-parametric data were determined by chi-squared tests. Analyses of primary and secondary outcomes are performed with the use of time-to-event methods according to the intention-to-treat principle. All analyses were two-sided, and $p<0.05$ was considered statistically significant. Statistical analyses were performed using Prism version 5 (GraphPad Software Inc., La Jolla, CA, USA).

5. Handling of Missing Data：

Omit the cases with missing data and analyze the remaining data.

2. Introduction and Rationale

2.1 Investigational product(s)

Powered muscle stimulator/ GMORE

GM300E

2.2 Animal and preclinical study data

Not applicable

2.4 Risks / benefits Assessment

1. In our preliminary study, relative high protein intake (1.2g/kg/day) for less than one month had no significant impact on renal function. We will follow serum creatinine level at least twice per week for monitoring.

2.5 Regulatory

This study will be conducted in compliance with the protocol approved by the Institutional Review Board, and according to Good Clinical Practice standards. No deviation from the protocol will be implemented without the prior review and approval of the IRB except where

it may be necessary to eliminate an immediate hazard to a research subject. In such case, the deviation will be reported to the IRB as soon as possible.

3. Objectives and Endpoints

3.1 Study Objectives:

3.1.1 Primary objective:

To evaluate the benefit of high protein supplementation and core muscle rehabilitation and trunk NMES intervention in weaning from ventilator

3.1.2 Secondary objectives:

The impact of renal function and digestion status after high protein intake

The benefit in nutrition status and diaphragm weakness and the correlation to weaning status

3.1.3 Other exploratory objectives (if any):

1.2 Study endpoints:

3.2.1 Primary endpoint:

Weaning rate

(weaning success defined as weaning from ventilator for 5 consecutive days)

3.2.2 Secondary endpoints:

In hospital mortality, Length of mechanical ventilator usage,

Length of ICU stay and total length of hospitalization,

Serum albumin level improvement, Creatinine clearance rate

3.2.3 Other exploratory endpoints (if any):

4. Study Design

4.1 Overall Design

Research principles and methods

Patients

Patients (age ≥ 20 years old) on mechanical ventilator for more than 21 days were recruited from

the respiratory care center (RCC). of Chang Gung Memorial Hospital in Taiwan.

The **inclusion criteria** were as follows:

1. age ≥ 20 year old
2. using mechanical ventilator for more than 21 days (including patients under tracheostomy or endotracheal tube)
3. stable clinical condition, without using inotropic agent
4. maximal inspiratory pressure (MIP) < 30mmHg
5. under enteral nutrition (EN) via NG tube.

The **exclusion criteria** were as follows:

1. Acute infection and sepsis (fever up to 38.5 degree)
2. Severe neuromuscular disease, or uncontrolled epilepsy
3. Bony fracture or DVT history
4. Wound over the abdomen
5. Congestive heart failure with EF < 40% or using pacemaker
6. BMI>35 kg/M^2, or severe edema
7. Patients with hepatic failure, rapid progressed malignancy, or pregnancy were also excluded.
8. Under parenteral nutrition (PN)
9. Use pacemaker

Patient's identification will be recorded as a study code, which will not be mentioned in the publication.

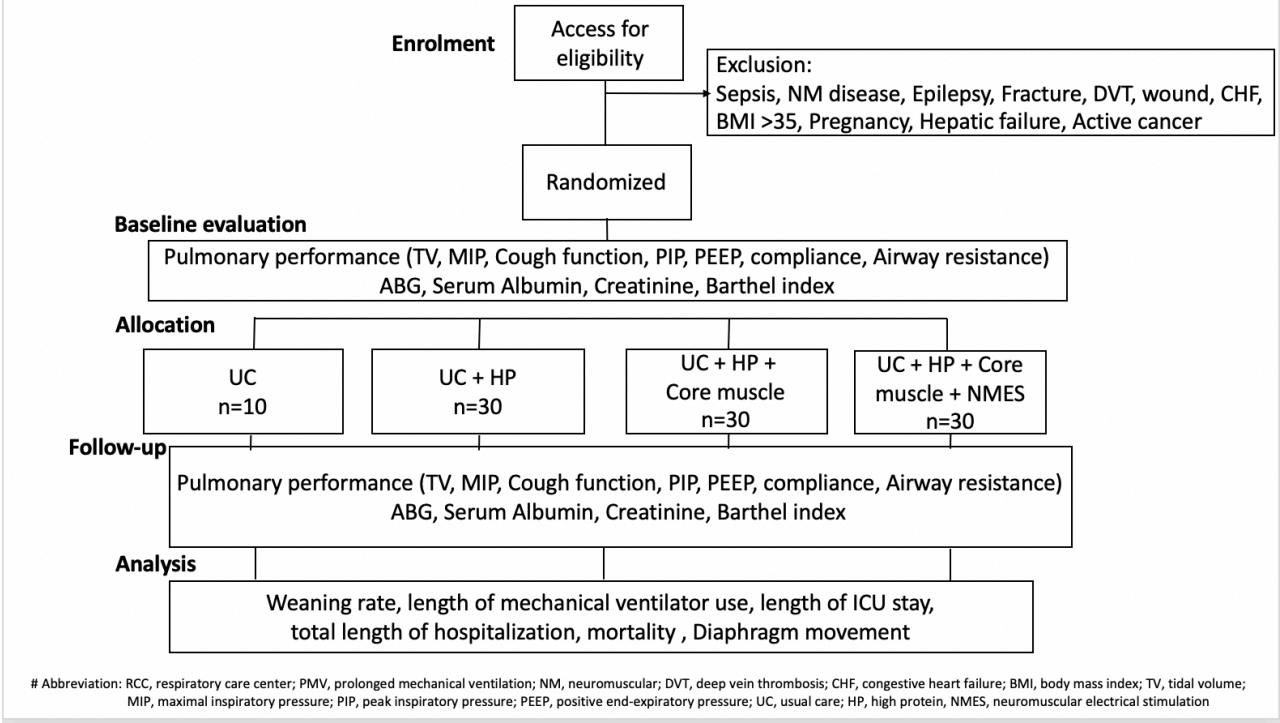
Study Protocol

Patients will be randomly assigned into four groups: (1) Usual care (UC), (2) UC + high protein diet (HP), (3) UC + HP + core muscle rehabilitation, (4) UC + HP + core muscle rehabilitation + neuromuscular electric stimulation (NMES). Ten patients will be assigned to UC group. Thirty patients will be included in another 3 group. Before subject recruitment, sequential sealed envelopes were prepared by an independent investigator (Chung-Chi Huang), with one chosen randomly by another investigator for each subject. The subjects were then assigned to each group according to the label in the envelope. The total daily caloric intake is based on the suggestion of nutritionist. The HP groups will maintain unchanged total daily caloric intake and increasing protein content to 1.5g/kg/day. The specific formulas were chosen for their different energy and

protein requirement (mainly Nutri-Aid , or NU-PEP HN , according to patients' digestion, and may add Whey-Aid if necessary (above formula are available in CGMH currently)). We will maintain the nutrition strategies for 21 days according to patients' weaning condition. Protein provision was not reduced in case of renal failure. Core muscle rehabilitation is sitting on bedside with or without aids, for 30 minutes, twice per day, 5 days per week, for 3 weeks. NMES was applied for 30 min, twice per day, 5 days per week, for 3 weeks via surface rectangular electrodes. Electrodes were placed on back designed to activate latissimus dorsi and abdominal wall designed to activate the transversus abdominis and internal and external oblique muscles as previously McCaughey et al described [37]. Electrical muscle stimulation was performed by using a commercial stimulator (GEMORE, GM300E, Taipei, Taiwan) with biphasic waves at a simulation frequency of 30 Hz and pulse width of 400s, cycling 2s on and 4s off. Electrical muscle stimulation intensity was gradually increased until a visible muscle contraction was observed (median 60 mA [range 50–65 mA].

The age, gender, smoking history, diagnosis, length of mechanical ventilator use, APACHE II will be recorded. The pulmonary performance, including tidal volume, maximal inspiratory pressure (MIP), cough function, peak inspiratory pressure (PIP), positive end-expiratory pressure (PEEP), lung compliance, airway resistance, blood gas analysis, Barthel index, serum albumin, creatinine, electrolytes will record before, during (twice per week) and after intervention. We also follow the outcome of hospitalization, including the weaning rate, mortality, length of mechanical ventilator use, length of ICU stay and total length of hospitalization. We will further evaluate the diaphragm movement. Ultrasound was performed at the end of exhalation (without stimulation) to measure the thickness of the diaphragm, twice per week of participation, and then weekly until RCC discharge. All measurements were taken from muscles on the right-hand side of the participant by the same assessor at all assessment sessions. For the diaphragm, the probe was placed parallel to the anterior axillary line in the intercostal space between the 9th and 10th rib and moved in the cranial and caudal directions until the pleural line was identified. From this point, the probe was moved approximately 1 or 2 intercostal spaces lower to identify the costal diaphragm in the zone of apposition. In all measurements, the probe was placed perpendicular to the skin. We will analyze the diaphragm movement, thickness and the correlation with successful weaning rate.

Flow Chart ：



Abbreviation: RCC, respiratory care center; PMV, prolonged mechanical ventilation; NM, neuromuscular; DVT, deep vein thrombosis; CHF, congestive heart failure; BMI, body mass index; TV, tidal volume; MIP, maximal inspiratory pressure; PIP, peak inspiratory pressure; PEEP, positive end-expiratory pressure; UC, usual care; HP, high protein, NMES, neuromuscular electrical stimulation

4.2 Number of Patients

Evaluable Number: 100

Enrolled Number: 80 (20% dropped out, or failure to finish study)

4.3 Schedule of Activities

Time-Event scheme :

Phase	Screening	Treatment			Treatment			Treatment			Follow-up		
Day	0-1	1~3	4±1	6~7	8~10	11±1	13~14	15~17	18±1	20~21	22~27	28±1	
	Baseline										Safety follow up visit/Final study visit		
In-hospital visit	X	X	X		X	X		X	X	X	X	X	
Screening/Administratiive/Safety													
ICF/assent form	X												
Inclusion/Exclusion criteria	X												
Medical and surgical history, demographics	X												
Physicl exam	X		X			X			X			X	
Vital signs	X	X	X			X			X			X	
Height and body weight	X		X			X			X			X	
Pulmonary performance (ventilator)	X		X			X			X			X	
Laboratory study	X		X			X			X			X	
Barthel index	X												
Echo for diaphragm evaluation	X		X			X			X			X	
Randomization/Administration													
Randomization	X												
Administration of high protein diet		X	X	X	X	X	X	X	X	X			
Administration of core muscle rehabilitation		X	X		X	X		X	X				
Administration of NMES		X	X		X	X		X	X				
Efficacy/Safety													
Weaning profile	X		X			X			X	X		X	
Creatinine clearance rate	X		X			X			X	X		X	
Weaning rate										X		X	
Length of mechanical ventilator use										X		X	
Length of ICU stay										X		X	
Length of hospitalization										X		X	
In hospital Mortality										X		X	

5. Study Population

5.1 Inclusion Criteria

The **inclusion criteria** were as follows:

1. age ≥ 20 year old
2. using mechanical ventilator for more than 21 days (including patients under tracheostomy or endotracheal tube)
3. stable clinical condition, without using inotropic agent
4. maximal inspiratory pressure (MIP) < 30mmHg
5. under enteral nutrition (EN) via NG tube.

5.2 Exclusion Criteria

The **exclusion criteria** were as follows:

1. Acute infection and sepsis (fever up to 38.5 degree)
2. Severe neuromuscular disease, or uncontrolled epilepsy
3. Bony fracture or DVT history
4. Wound over the abdomen
5. Congestive heart failure with EF < 40% or using pacemaker
6. BMI>35 kg/M^2, or severe edema
7. Patients with hepatic failure, rapid progressed malignancy, or pregnancy were also excluded.
8. Under parenteral nutrition (PN)
9. Use pacemaker

5.3 Withdrawal criteria

1. Return to ICU for unstable vital sign or any necessity of surgery.
2. Patient refuse further intervention
3. Physicians judge the patients' condition are not suitable for further intervention.

10. Treatments

6.1.Treatment Administration

1. High protein diet:

The HP groups will maintain unchanged total daily caloric intake and increasing protein content to 1.5g/kg/day. The specific formulas were chosen for their different energy and protein requirement (mainly Nutri-Aid , or NU-PEP HN , according to patients' digestion, and may add Whey-Aid if necessary (above formula are available in CGMH currently)).

We will maintain the nutrition strategies for 21 days according to patients' weaning condition. Protein provision was not reduced in case of renal failure.

2. Core muscle rehabilitation:

Sitting on bedside with or without aids, for 30 minutes, twice per day, 5 days per week, for 3 weeks.

3. Neuromuscular electrical stimulation (NMES):

NMES was applied for 30 min, twice per day, 5 days per week, for 3 weeks, via surface rectangular electrodes. Electrodes were placed on back designed to activate latissimus dorsi and abdominal wall designed to activate the transversus abdominis and internal and external oblique muscles as previously McCaughey et al described [37]. Electrical muscle stimulation was performed by using a commercial stimulator (GEMORE, GM300E, Taipei, Taiwan) with biphasic waves at a simulation frequency of 30 Hz and pulse width of 400s, cycling 2s on and 4s off. Electrical muscle stimulation intensity was gradually increased until a visible muscle contraction was observed (median 60 mA [range 50–65 mA].

The subjects in the control group received similar electrode placement and intervention duration, except that the stimulator power was off.

6.2. Concomitant Therapy

Not Applicable

7. Efficacy Assessments

The age, gender, smoking history, diagnosis, length of mechanical ventilator use, APACHE II will be recorded initially.

The pulmonary performance, including tidal volume, maximal inspiratory pressure (MIP), cough function, peak inspiratory pressure (PIP), positive end-expiratory pressure (PEEP), lung compliance, airway resistance, blood gas analysis, Barthel index, serum albumin, creatinine, electrolytes will record before, during (twice per week) and after intervention.

We also follow the outcome of hospitalization, including the weaning rate, mortality, length of mechanical ventilator use, length of ICU stay and total length of hospitalization.

8. Safety Assessments

We will follow serum creatinine level and creatinine clearance rate weekly.

During core muscle rehabilitation and NMES, vital signs (heart rate, blood pressure, respiratory rate) and parameters in ventilator (tidal volume, FiO2, peak airway pressure) will be monitored.

9. Adverse event reporting

We will report SAEs to the IRB of Chang Gung Medical Foundation according to the Serious Adverse Event Reporting Procedures and Guidelines as posted in the Clinical Trials Resource on the website of Chang Gung Medical Foundation IRB. SAE reports to the IRB should include the following information when calling the Medical Monitor:

- *Date and time of the SAE*
- *Date and time of the SAE report*
- *Name of reporter*
- *Call back phone number*
- *Affiliation/Institution conducting the study*
- *Protocol number*
- *Title of protocol*
- *Description of the SAE, including attribution to drug and expectedness*

9.1 Definitions and reports of Adverse Events

All adverse events that occur after the informed consent is signed (including run-in) must be recorded on the adverse event CRF (paper and/or electronic) whether or not related to study

agent. AE Data Elements including:

- AE reported date
- AE Verbatim Term
- CTCAE Term (v 5.1)
- Event onset date and event ended date
- Severity grade
- Attribution to study agent (relatedness)
- Whether or not the event was reported as a Serious Adverse Event (SAE)
- Action taken with the study agent
- Outcome of the event
- Comments

Identify the adverse event using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. The CTCAE provides descriptive terminology and a grading scale for each adverse event listed.

AEs will be assessed according to the CTCAE grade associated with the AE term. AEs that do not have a corresponding CTCAE term will be assessed according to their impact on the participant's ability to perform daily activities as follows:

Grade	Severity	Description
1	Mild	• Barely noticeable, does not influence functioning • Causing no limitations of usual activities
2	Moderate	• Makes participant uncomfortable, influences functioning • Causing some limitations of usual activities
3	Severe	• Severe discomfort, treatment needed • Severe and undesirable, causing inability to carry out usual activities
4	Life threatening	• Immediate risk of death • Life threatening or disabling
5	Fatal	• Causes death of the participant

The possibility that the adverse event is related to study drug will be classified as one of the

following: not related, unlikely, possible, probable, definite.

DEFINITION of Serious Adverse Events: ICH Guideline E2A and GCP of Taiwan define serious adverse events as those events, occurring at any dose, which meet any of the following criteria:

- Results in death
- Is life threatening (Note: the term life-threatening refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe).
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital abnormality/birth defect
- Events that may not meet these criteria, but which the investigator finds very unusual and/or potentially serious, will also be reported in the same manner.

9.2 Adverse event follow-up

All AEs, including lab abnormalities that in the opinion of the investigator are clinically significant, will be followed according to good medical practices and documented as such. Site staff should send follow-up reports as requested when additional information is available. Additional information should be entered on the IRB of Chang Gung Medical Foundation of SAE form in the appropriate format. Follow-up information should be sent to Chang Gung Medical Foundation IRB as soon as possible according to IRB's Serious Adverse Event Reporting Procedures and Guidelines.

10. Criteria for the termination of the trial

Not applicable.

11. Statistical Considerations

11.1 Sample size Determination

This is an exploratory research, we determine the enrolled number according to the

number of patients admitted to RCC annually.

Ten patients will be assigned to UC group.

Thirty patients will be included in another 3 group.

A total 100 patients will be enrolled.

11.2 Planned Statistical methods of analysis

Categorical variables were expressed as count and percentage. Parametric data were expressed as mean ± standard deviation (SD). A Student's t-test was used to compare parametric data. Fisher's exact test was used to compare non-parametric data. We used a receiver operating characteristic (ROC) curve to identify the optimal cut-off-value for the maximum multiplication of sensitivity and specificity. Logistic regression was used for multivariate analysis. Kaplan–Meier survival analysis was performed to assess weaning and survival outcomes. Statistical significance was set at a p-value < 0.05. Statistical analyses were performed using GraphPad Prism version 8 (GraphPad Software, La Jolla, CA, USA) and IBM SPSS Statistics 26 (SPSS, Chicago, IL, USA).

11.2.1 Efficacy analysis

Kaplan–Meier survival analysis was performed to assess weaning and survival outcomes.
Mann Whitney test was used for compare non-parametric data (weaning rate, length of ventilator use, length of ICU stay and length of hospitalization).

11.2.2 Safety analysis

Wilcoxon matched-pairs signed rank test was used to compare parametric data (difference of baseline and follow-up serum creatinine and creatinine clearance)

11.2.3 Additional analysis

Not applicable

11.2.4 The level of significance

A p-value less than 0.05 was considered significant and was denoted by * and p-value less than 0.01 was denoted by **.

11.3 Analysis Population

All subjects randomized.

11.4 Procedure for accounting for missing, unused and spurious data

The incomplete clinical data and sequencing data with poor quality will be excluded in the study.

12. Direct access to source data/documents

Investigators permit IRB to access to the source data of experiment for trial-related monitoring, audits and regulatory inspection.

13. Ethical considerations

This study will be conducted according to Taiwan and international standards of Good Clinical Practice for all studies. Applicable government regulations and Chang Gung Medical Foundation research policies and procedures will also be followed.

This protocol and any amendments will be submitted to the Chang Gung Medical Foundation Institutional Review Board (IRB) for formal approval to conduct the study. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB. The formal consent of a subject, using the IRB-approved consent form, will be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

14. Data handling and keeping

Clinical samples will be collected in Chang Gung Medical Foundation. The sequencing data will be stored in computers of laboratory with an electronic encryption. The clinical and source data can only be assessed by clinical doctors and investigators of the study.

15. Financing and Insurance

Apply for CMRP support. The funders had no role in the study design, data collection and analysis, decision to publish, or manuscript preparation.

16. References

Background

Ventilator-induced diaphragm dysfunction (VIDD)

Diaphragmatic function was an important respiratory muscle and play a major role in weaning success[1]. Various insults can weaken the diaphragm, but the most common seen in critically ill patients were the use of controlled mechanical ventilation (CMV)[1,2]. The ventilator assumes the entire work of breathing and the diaphragm is for all practical purposes inactive, can rapidly lead to atrophy and weakness of the muscle, termed ventilator-induced diaphragmatic dysfunction (VIDD) [3]. Use of mechanical ventilation modes in which the ventilator assists, but does not completely supplant, spontaneous diaphragmatic efforts can, to various degrees, attenuate VIDD [1,2]. Reynolds et al conducted a study in 18 pigs, use intermittent bilateral phrenic nerve pacing with to prevent the decline in diaphragmatic thickness, and to mitigate reductions in diaphragm muscle fiber cross-sectional area after 60 hours of mechanical ventilation. Maximal pressure generation by the diaphragm was not affected, but endurance appeared to be superior ($P = 0.055$) in the paced group[4]. Is phrenic nerve pacing the sole answer to the problem of VIDD? Further research warranted to prove it. Frequent reassessment of the patient's ability to resume spontaneous breathing and early use of partial-support MV are potential strategies to preserve diaphragm protein synthesis during prolonged MV [2]. Attenuate weakness of diaphragm is an important step in successful weaning.

Predictors for weaning from prolonged mechanical ventilation

Several studies have assessed predictors of weaning and extubation outcome in short-term mechanically ventilated patients, but there are only few studies on predictors of weaning from prolonged mechanical ventilation [5]. Female, obesity (BMI>30 kg/m^2), hypercapnia, higher tidal volume/ideal body weight were independent predictors for weaning failure [5,6]. The rapid shallow breathing index (RSBI) was introduced by Yang and Tobin in 1991. They found that there was a higher probability of weaning success if the RSBI was ≤ 105 and spontaneous breathing trials were successful [6-9]. In systematic review, Trivedi et al stated that the RSBI has moderate sensitivity and poor specificity for predicting extubation success and suggested that it should be a permissive

criterion to undergo a spontaneous breathing trial (SBT) for patients who are at intermediate pretest probability of passing an SBT [8].

Since the RSBI represented as respiratory rate/tidal volume (RR/TV), increasing tidal volume is quite essential in weaning patients. The factors involving in tidal volume including the VIDD, strength of inspiratory muscle and nutritional status.

Protein supplement strategy in patients with prolonged mechanical ventilation (PMV)

Critically ill patients undergo severe metabolic stress during which time a great amount of energy and protein is utilized in a variety of reactions essential for survival [10]. In non-septic critically ill patients, early high protein intake (1.2g/kg/d) was associated with lower mortality and early energy overfeeding with higher mortality [11]. Energy targets are a matter of debate for intensive care (ICU) patients. The optimal approach to feeding the critically ill, with increasing interest in the concept of intentional underfeeding to reduce metabolic stress while maintaining gut integrity [12]. In one post hoc–defined outcome, the full energy feeding group was observed to have a significantly higher likelihood of being discharged home with or without assistance with activities of daily living (68.3% versus 51.3%, P=0.04)[13]. Although not reaching statistical significance, full energy feeding survivors tended to show improved physical and cognitive function, with a greater proportion returning to employment. The full-feeding group also reported a significantly lower incidence of admission to physical rehabilitation facilities and trended towards lower new residence in healthcare facilities[14]. A linear reduction in energy target recommendation without changing the feed composition led to an unplanned and significant reduction in protein delivery, which was associated with a prolonged duration of ventilation and an extended hospital stay [15]. Thus, during the chronic ICU phase, higher protein/caloric targets should be provided preferably combined with exercise [16]. Zhang et al conducted a prospective trial discussing the effect of high protein supplement (2g/kg/d) on diaphragm atrophy and found that intensive nutrition treatment (INT) improved the diaphragm atrophy and muscle mass of critically ill patients receiving prolonged MV. There was no evidence that increasing protein to the target amount of 2.0 g/kg/d is related to improvement in clinical prognosis for patients receiving prolonged MV [17]. The optimal protein intake during critical illness is unknown. Conflicting results on nutritional support during the first week of ICU stay have been published. Koekkoek et al conducted a retrospective study focusing on the timing of protein intake among critically ill patients and found that lowest 6-month mortality was associated with patients receiving increasing protein intake from <0.8 g/kg/day on day 1-2 to 0.8-1.2 g/kg/day on day 3-5 and >1.2 g/kg/day after day 5 [18]. Timing of high protein

intake may be quite relevant in determining outcomes in ICU patients.

Rehabilitation in critically ill patients

Neuromuscular dysfunction in the ICU was common, including demonstrating with flaccid limbs, loss of reflexes, and delayed weaning from mechanical ventilation [19]. More than 50% of patients discharged from the ICU had developed ICU-acquired weakness, which was associated with death between ICU discharge and day-90 [20]. The risk factors for neuromuscular disorders and functional impairment were bed rest, corticosteroids, and neuromuscular blockers (NMBs). Early mobilization and rehabilitation are quite safe and feasible in patients who are critically ill, with potential benefits including improved physical functioning and decreased duration of mechanical ventilation, intensive care, and hospital stay [19].

Attenuate weakness of diaphragm is an important step in weaning. One potential treatment that may be useful as an additional form of therapy would be to add muscle-specific forms of exercise. Such therapies include various forms of volitional or electrically induced exercise directed at various limb and respiratory muscles. In particular, inspiratory muscle training has been shown to significantly improve diaphragm function and increase the maximal inspiratory pressure [21]. Inspiratory muscle training had been proved to be beneficial in patients with COPD and bronchiectasis [21,22]. Elkins et al systematically reviewed 10 studies involving 394 participants and showed that the inspiratory muscle training significantly improved maximal inspiratory pressure, the RSBI and weaning success. The significant benefits were also reported for the time spent on non-invasive ventilation after weaning and length of stay in hospital. Weaning duration decreased in the subgroup of patients with known weaning difficulty [23].

Furthermore, arranging patient-specific rehabilitation program based upon each patients' functional level, also increased the weaning rate in PMV patients. The multimodal rehabilitation program (MRP) is a progressive, patient-specific rehabilitation program, categorized into bed dependent, chair dependent and ambulatory groups. MRP improves strength, physical function and mobility to usual physical therapy in ICU acquired weakness and was associated with greater weaning success and discharge home than UC alone [24]. Schreiber et al conducted a retrospective analysis on 1,313 consecutive patients admitted to a weaning unit over a 15-y period for prolonged mechanical ventilation. Subjects underwent a program of intensive physiotherapy organized in 4 incremental steps: (1) the ability to maintain a sitting position on the edge of the bed and to perform cycling against resistance in bed; (2) the ability to maintain a sitting position in a chair and regain standing posture; (3) the ability to start active transfer from bed to chair and to walk with the

aid of a rollator and physiotherapists; and (4) the achievement of walking autonomy, with or without the aid of a stick and/or a person. Stepwise logistic regression analysis showed that achievement of > 2 physiotherapy steps was the main predictor of successful weaning [25]. This indicated that it is possible that whole body exercise (eg, walking in mechanically ventilated patients) may also improve diaphragm strength or endurance.

Neuromuscular electrostimulation (NMES)

NMES had played an important role in stroke patients. NMES is also useful for strengthening peripheral muscles, augment gains in body weight and quality of life in patients with cystic fibrosis with severe pulmonary obstruction [26]. NMES could improve exercise capacity and reduce perceived sensation of dyspnea during exercise in patients with COPD, but not to be recommended as an effective alternative training modality in the rehabilitation of stable COPD patients[27-29]. Home-based NMES as an add-on to PR did not result in further improvements in subjects with severe to very severe COPD [30,31]. The use of NMES was suggest in patients who were unable to engage with conventional pulmonary rehabilitation [29]. However, the majority of NMES were performed in leg muscles. Zayed et al conducted a systematic review and demonstrated that NES combined with usual care was not associated with significant differences in global muscle strength, ICU mortality, duration of MV, or ICU length of stay in comparison with usual care alone in critically ill patients [32]. But Leite et al showed electrical stimulation of quadriceps had best outcomes for peripheral muscle strength compared with controls or electrical stimulation of diaphragm among mechanically ventilated critically ill subjects and promoted functional independence and decreased length of hospitalization [33]. The effects of limbs NMES remained controversial.

Recently, WB-EMS is proved to be a safe and attractive method for increasing muscle mass and functional capacity in this cohort of women 70+ with sarcopenic obesity [34]. Core muscle strengthening combined with trunk NMES improved on trunk balance in post-stroke patients [35] and the effects were similar in abdominal and back muscles [36]. Since the diaphragm played an important role in respiration and weaning. McCaughey et al preformed a prospective study in 20 critically ill mechanically ventilated participants. Electrodes were placed posteriorlaterally over the abdominal wall designed to activate the transversus abdominis and internal and external oblique muscles. The active group received abdominal FES at an intensity that caused a strong visible muscle contraction (median 60 mA [range 50–65 mA]), with a frequency of 30Hz and a pulse width of 350 μs. The stimulation current in the control group was set at 10 mA (possible sensation

but no muscle contraction), with a frequency of 10Hz and a pulsewidth of 350 μs. There appeared to be no between-group thickness changes of the rectus abdominis, diaphragm or combined lateral abdominal muscles. However, ICU length of stay and ventilation duration appeared to be shorter in the intervention compared to the control group [37]. Cho et al conducted a prospective trial discussing the effects of NMES in different frequencies and demonstrated that 50 Hz produced best muscle thickness increases [38]. Sewa et al revealed that functional electrical stimulation can be safely delivered to human abdominal muscles without causing vital sign abnormalities. It was also found that the appropriate intensity level of electrical stimulation for achieving effects on respiratory flow while also minimizing pain is 60–80 mA[39]. The effects of NMES on abdominal muscles and diaphragm were not clear.

Overall, the effects of protein nutrition, exercise, NMES appear to be beneficial in critically ill patients. However, studies into the differential effects of protein nutrition and/or exercise combined with trunk NMES, and optimization of their combined use, have not been performed yet [40].

Aims

The aim of this study is to investigate the outcomes of combined high protein supplementation and NMES on truck muscles in patients with prolonged mechanical ventilation.

Importance

Analyzing the role and timing of high protein supplementation and trunk NMES intervention will provide a more precise treatment strategy in future for weaning in PMV patients.

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