ORIGINAL ARTICLE

Effect of High Protein Normocaloric Nutrition on Skeletal Muscle Wasting in Critically Ill Mechanically Ventilated Patients: A Randomized Double-blind Study

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Abstract

Background and aims: Muscle wasting in critically ill patients is associated with poor outcomes. During intensive care unit (ICU) stay, delivering appropriate nutritional support helps minimize muscle loss. We sought to evaluate the impact of high-dose protein on muscle thickness and cross-sectional area (CSA), as well as to track changes in muscle echogenicity and pennation angle (PA) using bedside ultrasound in this population.

Patients and methods: We conducted a randomized, prospective, double-blind trial in which 30 patients mechanically ventilated for more than 48 hours and receiving enteral feed were enrolled. Patients were divided into two groups, and all patients received enteral feeds with total calories of about 25 kcal/kg/day. In the high-protein feed (HPF) group, patients were targeted to receive 1.5 gm/kg/day of protein, whereas in the standard feed (SF) group, patients received 1 gm/kg/day of protein. After ICU admission, muscle thickness, CSA, echogenicity, and PA were measured in all mechanically ventilated patients on days 1, 3, 5, and 7 using bedside ultrasound. The right lower limb vastus lateralis (VL) and the medial head of the gastrocnemius were investigated.

Results: We found a progressive decrease in muscle mass from day 1 to day 7 in all patients. Our study showed that muscle thickness and CSA were significantly higher in the HPF group than the SF group over 7 days, whereas muscle echogenicity and PA changes were not statistically significant.

Conclusion: High-protein feeds prevent muscle wasting in critically ill patients compared to patients receiving SFs during the first week of ICU stay. The qualitative muscle parameters, like muscle echogenicity and PA changes, were not significant.

Keywords: Critical illness, High protein diet, Muscle wasting, Nutrition.

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Highlights

Skeletal muscle wasting in critically ill patients is common and leads to poor outcomes. In this study, the effect of a high-protein diet was compared with a standard-protein diet and was found to have a decrease in muscle wasting in high-protein diet as assessed by quantitative [muscle thickness and cross-sectional area (CSA)] and qualitative [muscle echogenicity and pennation angle (PA)] muscle parameters measured by ultrasound measurement. However, the qualitative parameters were not significant.

Introduction

Clinically detectable weakness in critically ill patients who are not having any etiology or condition extrinsic to underlying critical illness is called as intensive care unit-acquired weakness (ICU-AW).[1] Skeletal muscle weakness contributes significantly to physical and functional disability, which also negatively impacts mortality, ventilator, and ICU-free days.[2,3] The extent of skeletal muscle wasting directly correlates with the severity of organ dysfunction in critically ill patients, especially those with acute respiratory distress syndrome (ARDS).[4] During the acute phase of illness, an imbalance favoring muscle protein degradation over synthesis leads to a persistent catabolic state in critically ill individuals.[2,5,6] The protein dose, source of protein, and timing of ingestion can further influence skeletal muscle gains with exercise.[7] Among the types of proteins used in nutritional supplements, whey protein is more effective as it is a rich leucine source. Leucine is essential for muscle building, as the rate of protein production is proportional to the leucine content of the meal.[8] Conventionally, ICU-AW is identified through the use of clinical examination.[9] However, there is often a delay in diagnosing critical illness myopathy due to the patient's neurological status on clinical examination.[10,11] Diagnostic modalities like muscle biopsy and nerve conduction studies are employed to detect ICU-AW. However, these techniques are both invasive and costly. Timely recognition of critical illness myopathy is essential, as the majority of muscle alterations manifest within the initial 10 days of critical illness.[2]

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Ultrasonography (USG) is commonly utilized as a noninvasive modality for assessing and quantifying skeletal muscle mass.[12] Qualitative alterations in muscle architecture can likewise be evaluated through assessments of echogenicity and PA using USG.[13] The echogenicity of muscles on ultrasound is usually low, as the healthy tissue contains little fibrous content, which is mainly responsible for ultrasonic reflection.[14,15]

Muscle echogenicity also increases in ICU patients.[16] The PA refers to the angle at which muscle fibers insert into the aponeurosis, reflecting muscle fiber orientation and architecture. It is directly correlated with muscle strength and higher the PA, the more the contractile material is arranged within a given volume.[17] Thus, we decided to use B-mode ultrasound to characterize the differences in muscle mass, muscle echogenicity, and fascial characteristics such as the PA. In this prospective clinical study, we evaluated the impact of high-dose protein feed on quantitative and qualitative alterations in the vastus lateralis (VL) and gastrocnemius muscles. These parameters were assessed using USG in intubated ICU patients over the initial 7 days of admission.

Patients and Methods

This investigation was conducted as a prospective, randomized, double-blind trial within the ICU setting of the All India Institute of Medical Sciences, New Delhi, in the Department of Anaesthesiology, Pain Medicine, and Critical Care. After recieving institutional ethical committee approval (Approval no. = NK/ 1607/MD/10089-90) and CTRI clearance (CTRI/2020/02/023512), patients were enrolled. Written informed consent was procured from the relative or next of kin of the patient prior to enrollment in this investigation. At the time of study inclusion, all participants were receiving nutritional support via enteral feeding.

The study population consisted of patients admitted to the ICU who met the following inclusion criteria: enrollment on the day of ICU admission, post-intubation status, age between 18 and 70 years inclusive, and a clinical expectation of requiring endotracheal intubation for a duration exceeding 48 h. Patients who underwent successful extubation within the initial 7-day period following intubation were subsequently excluded from the final analysis. Individuals not meeting these predefined inclusion criteria were not eligible for study enrollment.

Patients were excluded from participation if informed written consent could not be obtained from their relative or next of kin, or if they had any of the following conditions: pregnancy, history of neurological problems, neuromuscular diseases or muscular dystrophies, vascular insufficiency or amputation of the limbs, prolonged immobility before ICU admission, unable to tolerate enteral feed, history of long-term steroids (>1 month) and trauma.

For each enrolled participant, the following demographic and clinical data were prospectively collected and recorded: Age (years), sex (male/female), weight (kilograms), pre-existing history of cardiac and pulmonary comorbidities, Nutrition Risk in Critically Ill (NUTRIC) score, Acute Physiology and Chronic Health Evaluation (APACHE) II score, arterial partial pressure of oxygen to inspired fractional oxygen ratio (PaO_2/FiO_2) on the first day of ICU admission, length of stay in the ICU (days), duration of mechanical ventilation (days), daily vasopressor requirements (expressed as a standardized dose or units/kg/hour) for the initial 7 days of ICU admission, and daily Sequential Organ Failure Assessment (SOFA) scores from day 1 to day 7. Data regarding the total length of hospital stay (days), discharge destination (e.g., home, rehabilitation facility, long-term care), and in-hospital mortality were retrieved from the hospital's electronic health record system.

Enteral feeding was started as soon as the patient was fully resuscitated, usually within 24 hours of ICU admission. The standard feed (SF) was prepared and provided to us by the dietician according to the study's requirement. The high-protein diet was prepared by the treating nurse, as per the allocation, by adding extra protein powder to the SF. Two different regimens of feed were assigned by randomization. Enrolled patients received either high-protein feed (HPF), which contained 25 kcal/kg/day of calorie with the protein of 1.5 gm/kg/day or SF, which included 25 kcal/kg/day of calorie with a 1 gm/kg/day protein.

Skeletal muscle characteristics were evaluated using USG, with the acquisition of both quantitative and qualitative data. Longitudinal B-mode ultrasound imaging of the VL and medial gastrocnemius (MG) muscles was performed on all enrolled patients on days 1 (within 24 hours of ICU admission), 3, 5, and 7. A portable ultrasound device (FUJIFILM SonoSite Edge®, Bothell, WA) equipped with a 6–12 MHz linear array transducer was utilized for all examinations. Baseline image optimization settings (e.g., contrast, gain) were maintained consistently across all assessments, with the exception of image depth, which was adjusted on an individual basis to ensure complete visualization of the muscle architecture.

Ultra-sonographers were blinded to the clinical and nutrition parameters. All ultrasound examinations are done by experienced researchers who have been using muscle ultrasound for >2 yr.

For scanning the VL, patient positioning was done by elevating the head end to 45° while keeping the legs flat on the bed. From the femoral lateral condyle, the measurement was taken 10 cm proximally, along both the long and short axes of the muscle to measure muscle thickness and CSA (Fig. 1A).

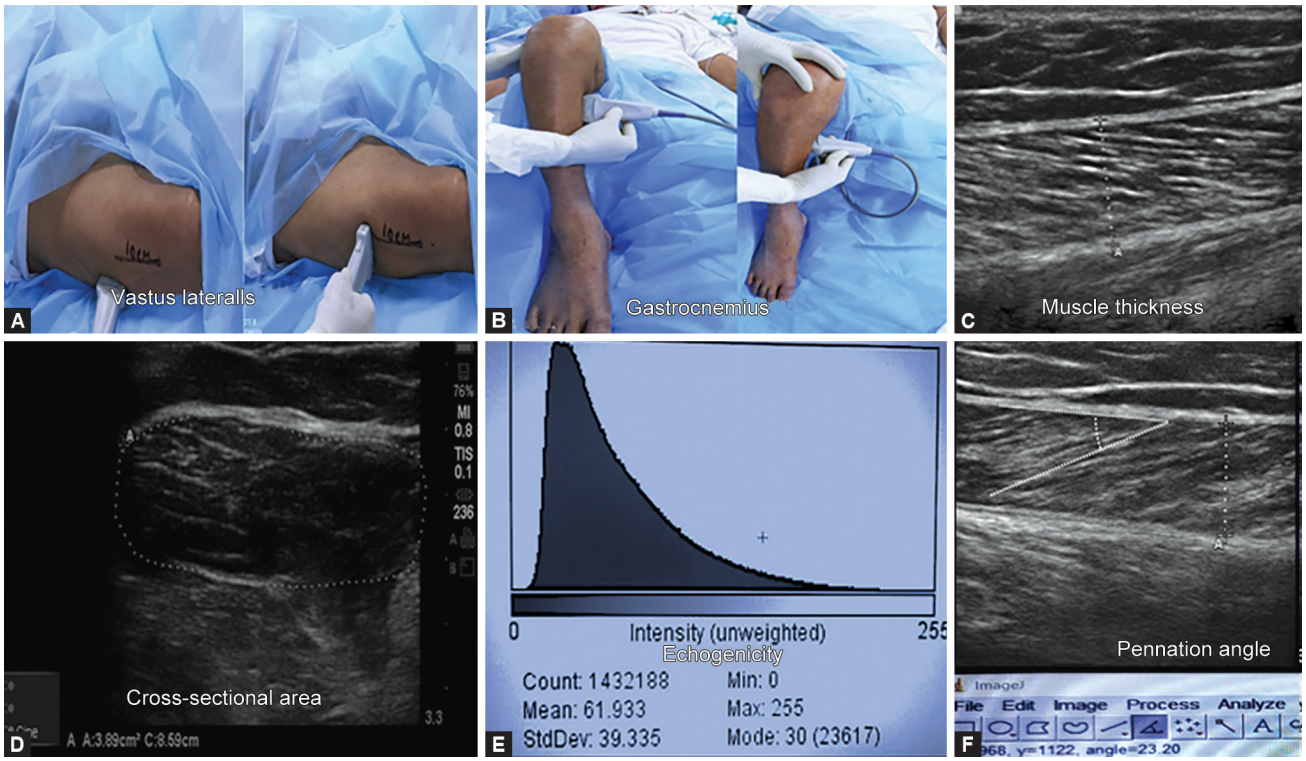
The optimal view of the gastrocnemius was done by knee flexion with the foot placed flat on the bed. Muscle thickness was measured at the point of maximal muscle belly diameter. Cross-sectional area was determined by tracing the muscle fascia along both the longitudinal and transverse planes of the ultrasound probe (Fig. 1B). The depth was adjusted to visualize both the aponeuroses along with muscle's fascicles. During the examination, all the images of muscles were saved in the ultrasound machine. Muscle thickness and CSAs are measured using the caliper and manual tracing inbuilt software of the ultrasound machine. For the measurement of echogenicity and PA, the saved images were exported to a laptop. The echogenicity and PA were measured using the freely available "ImageJ software" (version 1.47, National Institutes of Health, USA).

Muscle thickness (cm) was measured as the distance between the superficial and deep aponeuroses in each image (Fig. 1C)

The CSA measurement of the muscle was obtained through manual tracing of the muscle borders, and the measurements were recorded in centimeter square (Fig. 1D).

Muscle echogenicity was calculated post-image acquisition in terms of the mean grayscale value on a scale from 0 to 255 using grayscale histogram analysis with ImageJ software (version 1.47, National Institutes of Health, USA) (Fig. 1E).

The PA was taken as the angle between the fascicle and the superficial aponeurosis using ImageJ software (version 1.47, National Institutes of Health, USA) (Fig. 1F).



Figs 1A to F: Represent images of VL and gastrocnemius. (A) Vastus lateralis; (B) Gastrocnemius; (C) Muscle thickness; (D) Cross-sectional area; (E) Echogenicity; (D) Pennation angle

Categorical variables are presented as frequencies and percentages. Continuous variables are presented as mean ± standard deviation or median and interquartile range, as appropriate, based on the assessment of data normality using the Kolmogorov–Smirnov test.[1] For comparisons of quantitative variables, the independent *t*-test or the non-parametric Mann–Whitney *U* test was employed based on the normality of the data distribution. Qualitative variables were compared using the chi-square test or Fisher's exact test where applicable. The correlation between the NUTRIC score, day 1 PaO_2/FiO_2 ratio, day 1 SOFA score, and the percentage change in muscle size was evaluated using Spearman's rank correlation coefficient. A *p*-value of less than 0.05 was considered to indicate statistical significance. Data management and analysis were performed using Microsoft Excel spreadsheets and the Statistical Package for the Social Sciences (SPSS) version 21.0 (IBM Corp., Armonk, NY, USA).

Results

Of the 86 critically ill, mechanically ventilated patients evaluated for eligibility in this prospective investigation, 30 patients met the inclusion criteria and underwent randomization (Fig. 2). The final statistical analysis was performed on this cohort of 30 patients, whose baseline demographic and clinical characteristics are summarized in Table 1. The median age of the study participants was 36 years. At the time of admission, all enrolled patients were classified as having a good nutritional status (mean NUTRIC = 2.1), and most admission diagnoses were pneumonia (43.3%). No significant difference was found between patients of HPF and SF.

Results concerning muscular changes in the VL throughout the study are reported in Table 2. Muscle mass decreased irrespective

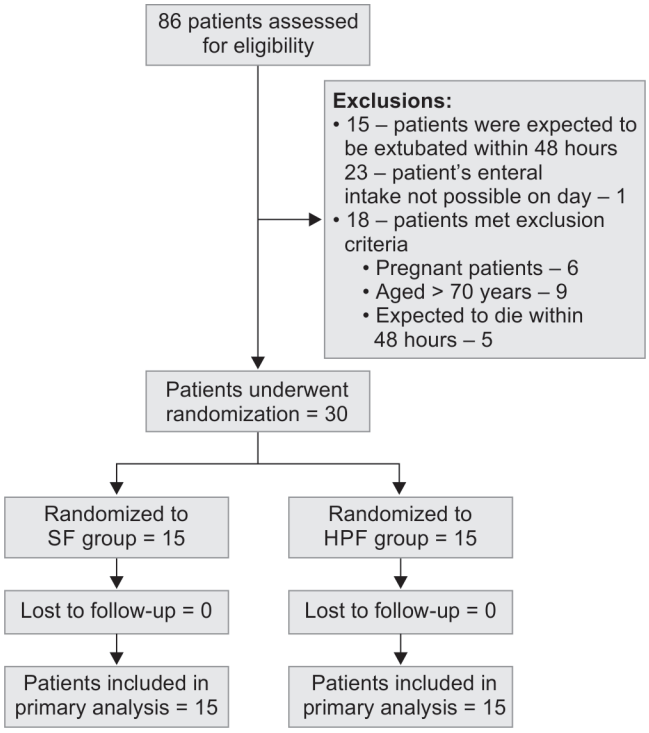


Fig. 2: Flow participants in the study

of protein supplement during the ICU stay in all patients. Muscle thickness was reduced significantly in the SF group (29.46%)

Table 1: Baseline characteristics of study population

Parameters	*HPF group*	*SF group*	*Total*	*p-value*
Male/Female	9 (60%)/6 (40%)	8 (53.33%)/7 (46.67%)	17 (56.67%)/13 (43.33%)	0.713
Age (years)	35.8 ± 13.73	36.6 ± 13.9	36.2 ± 13.58	0.884
Weight (kg)	63.6 ± 8.84	63.53 ± 8.98	63.57 ± 8.76	0.983
Septic shock	11 (73.3%)	10 (66.7%)	21 (70%)	1
Comorbidities	6 (40%)	6 (40%)	12 (40%)	1
Diagnosis				
AFI	6 (40%)	3 (20%)	9 (30%)	
Meningoencephalitis	3 (20%)	0 (0%)	3 (10%)	0.106
Pneumonia	5 (33.3%)	8 (53.33%)	13 (43.30%)	
Urosepsis	1 (6.67%)	4 (26.7%)	5 (16.7%)	
SOFA	7 ± 2.85	8.87 ± 2.72	7.93 ± 2.13	0.077
PaO_2/FiO_2	227.2 ± 61.68	204.13 ± 50.06	215.66 ± 62.3	0.167
APACHE-II	15.73 ± 7.28	17.8 ± 5.09	16.77 ± 6.26	0.375
NUTRIC	2.07 ± 1.58	2.13 ± 1.19	2.1 ± 1.37	0.831
MV-NOD (days)*	10.8 ± 6.05	11.47 ± 6.14	11.47 ± 6	0.767
LOIS (days)**	13.06 ± 6.77	15.93 ± 10.65	14.5 ± 8.89	0.387

*MV-NOD, mechanical ventilation number of days; **LOIS, length of ICU stay

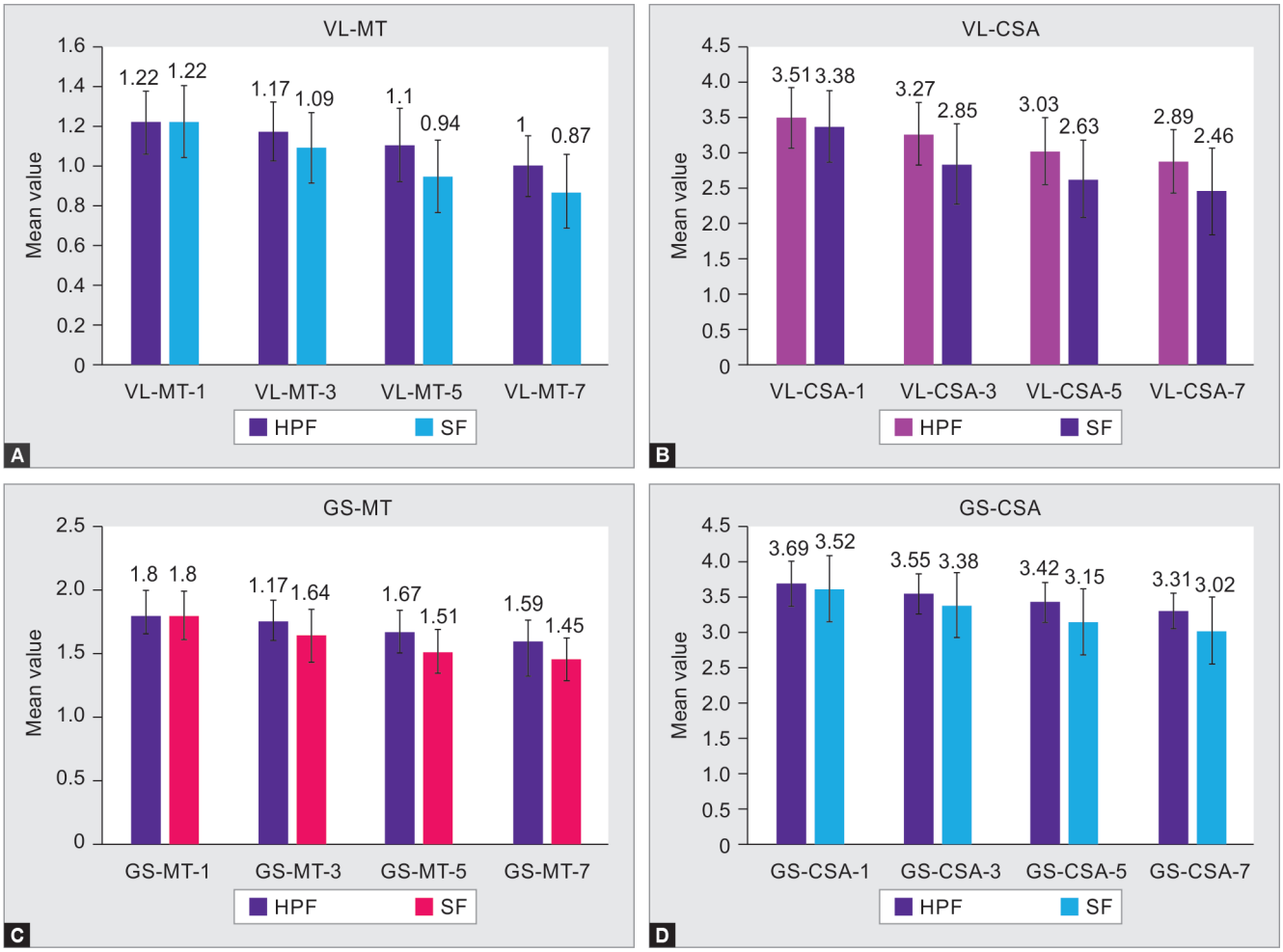
Table 2: Muscle wasting in VL on days 1, 3, 5, and 7

	HPF		*SF*		*p-value*	
Vastus lateralis	*Mean ± SD*	*Mean % change from Baseline ± SD*	*Mean ± SD*	*Mean % change from Baseline ± SD*	*HPF vs SF*	*% change between HPF vs SF*
VL-MT-1	1.22 ± 0.16	–	1.22 ± 0.18	–	0.983	–
VL-MT-3	1.17 ± 0.15	4.29 ± 4.79	1.09 ± 0.18	10.64 ± 10.63	0.196	0.008
VL-MT-5	1.10 ± 0.18	10.48 ± 7.95	0.94 ± 0.18	22.60 ± 8.20	0.038	0.001
VL-MT-7	1.00 ± 0.15	18.01 ± 7.84	0.87 ± 0.19	29.46 ± 9.86	0.039	0.003
VL-CSA-1	3.51 ± 0.43	–	3.38 ± 0.5	–	0.427	–
VL-CSA-3	3.27 ± 0.44	7.08 ± 4.56	2.85 ± 0.57	16.09 ± 6.87	0.034	<0.001
VL-CSA-5	3.03 ± 0.47	13.20 ± 6.09	2.63 ± 0.54	22.65 ± 7.68	0.028	0.002
VL-CSA-7	2.89 ± 0.45	18.02 ± 6.28	2.46 ± 0.62	27.81 ± 11.73	0.040	0.015
VL-ECH-1	50.53 ± 10.41	–	51.57 ± 10.74	–	0.789	–
VL-ECH-3	57.56 ± 10.53	15.63 ± 18.19	58.15 ± 10.51	13.71 ± 7.90	0.881	0.787
VL-ECH-5	62.82 ± 10.84	26.77 ± 21.43	62.33 ± 10.44	24.31 ± 10.75	0.896	0.917
VL-ECH-7	66.89 ± 98.3	35.59 ± 23.80	68.74 ± 11.32	35.03 ± 12.98	0.635	0.654
VL-PA-1	19.74 ± 3.14	–	19.71 ± 3.53	–	0.924	–
VL-PA-3	17.19 ± 3.27	12.93 ± 8.67	16.91 ± 3.51	14.44 ± 6.58	0.824	0.373
VL-PA-5	14.88 ± 3.17	24.44 ± 11.19	14.78 ± 3.27	25.35 ± 6.37	0.936	0.724
VL-PA-7	13.63 ± 3.25	30.74 ± 9.49	13.33 ± 2.83	32.49 ± 6.24	0.770	0.724

compared to the HPF group (18.01%) in the VL muscle at day 7 ($p = 0.003$), and an all-time point, this significant difference was observed (Fig. 3). Similarly, the CSA percentage decrease in the gastrocnemius was less in the HPF than in the SF group during the ICU stay, with a statistically significant difference among all time points between day 1 and day 7 (all $p < 0.05$). In the VL muscle, echogenicity progressively increased in all the patients, which was not significant. Similarly, the PA was decreased in all the patients, which was not significant between the groups ($p > 0.05$).

Results concerning muscular changes in the gastrocnemius are reported in Table 3. Muscle thickness and CSA decreased in the gastrocnemius muscle significantly in the SF group (MT = 19.58%, CSA = 16.64%) than the HPF group (MT = 12.49%, CSA = 9.97%) at day 7 [MT($p = <0.026$), CSA ($p = 0.046$)]. At all-time points, these changes were observed ($p = <0.05$). Echogenicity and the observed changes in the PA of the gastrocnemius muscle did not reach statistical significance between the HPF and SF groups at all time points of the study. There was no changes in the qualitative parameters in all the patients, in both the muscles during first 7 days of ICU stay.

We had 15 patients in each of the HPF and SF groups. There was no significant correlation of NUTRIC score, P/F ratio, and SOFA



Figs 3A to D: Quantitative muscle changes in VL and gastrocnemius muscle

Table 3: Muscle wasting in MG on days 1, 3, 5, and 7

	HPF		*SF*		*p-value*	
Gastrocnemius (GS)	*Mean ± SD*	*Mean % change from baseline ± SD*	*Mean ± SD*	*Mean % change from baseline ± SD*	*HPF vs SF*	*% change between HPF vs SF*
GS-MT-1	1.8 ± 0.2	–	1.8 ± 0.19	–	0.790	–
GS-MT3	1.75 ± 0.17	3.99 ± 2.94	1.64 ± 0.21	9.04 ± 5.68	0.140	0.001
GS-MT-5	1.67 ± 0.17	8.41 ± 3.40	1.51 ± 0.17	15.66 ± 6.72	0.021	0.001
GS-MT-7	1.59 ± 0.17	12.49 ± 3.10	1.45 ± 0.17	19.58 ± 6.58	0.026	0.001
GS-CSA-1	3.69 ± 0.32	–	3.62 ± 0.47	–	0.648	–
GS-CSA-3	3.55 ± 0.28	3.58 ± 2.40	3.38 ± 0.46	6.56 ± 2.28	0.237	<0.001
GS-CSA-5	3.42 ± 0.25	6.94 ± 3.48	3.15 ± 0.46	13.15 ± 3.65	0.051	<0.001
GS-CSA-7	3.31 ± 0.26	9.97 ± 3.91	3.02 ± 0.47	16.64 ± 4.71	0.046	<0.001
GS-ECH-1	40.66 ± 6.94	–	41.17 ± 6.13	–	0.833	–
GS-ECH-3	46.17 ± 3.07	14.42 ± 7.85	47.64 ± 5.33	16.47 ± 7.58	0.485	0.373
GS-ECH-5	51.02 ± 6.56	26.60 ± 9.73	52.7 ± 5.38	29.04 ± 9.55	0.451	0.494
GS-ECH-7	55.38 ± 6.75	37.73 ± 13.59	57.47 ± 6.02	40.74 ± 11.09	0.376	0.395
GS-PA-1	22.53 ± 2.43	–	22.54 ± 2.5	–	0.993	–
GS-PA-3	20.17 ± 2.53	10.54 ± 4.97	20.16 ± 2.44	10.53 ± 3.77	0.995	0.885
GS-PA-5	18.14 ± 2.62	19.34 ± 9.03	18.19 ± 1.91	19.12 ± 4.81	0.954	0.633
GS-PA-7	16.71 ± 2.55	25.57 ± 10.11	16.31 ± 1.86	27.55 ± 4.15	0.623	0.330

Table 4: Correlation of NUTRIC, PaO_2/FiO_2 ratio, SOFA score with muscle wasting

Variables	*HPF*	*NUTIRIC*	*P/F ratio*	*SOFA–1*	*SF*	*NUTIRIC*	*P/F ratio*	*SOFA–1*
VL MT-7	Correlation coefficient	0.181	–0.116	–0.088	Correlation coefficient	0.208	0.123	0.127
	p-value	0.518	0.679	0.753	*p*-value	0.456	0.660	0.650
	N	15	15	15	*N*	15	15	15
VL CSA-7	Correlation coefficient	0.250	–0.288	0.231	Correlation coefficient	0.433	–0.369	0.289
	p-value	0.367	0.296	0.406	*p*-value	0.106	0.175	0.296
	N	15	15	15	*N*	15	15	15
VL ECHO-7	Correlation coefficient	0.400	0.104	–0.428	Correlation coefficient	0.060	–0.423	–0.249
	p-value	0.138	0.712	0.111	*p*-value	0.830	0.115	0.389
	N	15	15	15	*N*	15	15	15
VL PA-7	Correlation coefficient	0.076	–0.195	0.216	Correlation coefficient	0.446	0.143	0.208
	p-value	0.785	0.484	0.437	*p*-value	0.094	0.609	0.456
	N	15	15	15	*N*	15	15	15
GS MT-7	Correlation coefficient	0.378	0.514	0.171	Correlation coefficient	0.189	–0.429	0.249
	p-value	0.163	0.051	0.540	*p*-value	0.498	0.110	0.370
	N	15	15	15	*N*	15	15	15
GS CSA-7	Correlation coefficient	–0.380	0.118	–0.427	Correlation coefficient	0.225	–0.306	0.123
	p-value	0.162	0.674	0.111	*p*-value	0.419	0.265	0.661
	N	15	15	15	*N*	15	15	15
GS ECH-7	Correlation coefficient	–0.457	0.374	–0.337	Correlation coefficient	–0.557	0.212	0.455
	p-value	0.08	0.168	0.218	*p*-value	0.030	0.446	0.088
	N	15	15	15	*N*	15	15	15
GS PA-7	Correlation coefficient	–0.057	0.188	–0.221	Correlation coefficient	0.169	0.339	–0.112
	p-value	0.838	0.501	0.427	*p*-value	0.546	0.216	0.688
	N	15	15	15	*N*	15	15	15

Table 5: Association of comorbidities and muscle wasting

	HPF		*SF*		*p-value*	
		% change from baseline		*% change from baseline*		*% change between*
Variables	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*	*HPF vs SF*	*HPF vs SF*
VL-MT-7	0.95 ± 0. 09	17.21 ± 8.2	0.94 ± 0.21	22.65 ± 13.0	0.972	0.406
VL-CSA-7	2.80 ± 0.43	18.36 ± 6.3	2.59 ± 0.76	20.29 ± 13.5	0.568	0.758
WECH-7	66.0 ± 8.1	18.29 ± 8.8	66.2 ± 7.0	33.7 ± 12.9	0.970	0.036
VL-PA-7	11.4 ± 1.8	35.93 ± 9.0	12.9 ± 3.2	30.19 ± 5.3	0.319	0.205
GS-MT-7	1.64 ± 0.13	14.26 ± 3.2	1.38 ± 0.23	–24.49 ± 6.9	0.038	0.008
GS-CSA-7	3.22 ± 0.19	8.47 ± 1.7	3.13 ± 0.49	–14.72 ± 2.5	0.685	<0.001
GS-ECH-7	56.68 ± 8.0	31.94 ± 12.5	57.97 ± 4.9	37.19 ± 9.2	0.743	0.427
GS-PA-7	16.33 ± 2.2	–26.18 ± 11.3	15.99 ± 2.0	–26.90 ± 3.6	0.785	0.885

score on the first day, with change in muscle mass expressed in percentage between two groups (Table 4). Six patients had comorbidities in each of the HPF and SF groups. In the VL muscle, the percentage change in echogenicity was significantly greater in the SF group (33.2%) compared to the HPF group (18.29%) ($p < 0.05$) (Table 5).

Eleven patients in the HPF and 10 patients in the SF groups had septic shock during ICU admission. In patients with septic shock, VL wasting was seen in both groups, but it was not statistically significant ($p > 0.05$), whereas in gastrocnemius, muscle thickness (HPF vs SF: 13.50 ± 3.0 vs 22.84 ± 7.2, $p = 0.001$) and CSA (HPF vs SF: 10.86 ± 4.6 vs 16.43 ± 5.5, $p = 0.021$) percentage changes were higher in SF group and were statistically significant (Table 6).

Discussion

This study employed USG to assess the impact of high-dose protein administration on both quantitative and qualitative alterations in skeletal muscles of mechanically ventilated patients during the initial 7 days of their ICU stay. Quantitative muscle changes were evaluated through serial measurements of muscle thickness and CSA in the VL and gastrocnemius muscles. Notably, the

Table 6: Association of septic shock and muscle wasting

	HPF		*SF*		*p-value*	
Variables	*Mean ± SD*	*% Change from baseline Mean ± SD*	*Mean ± SD*	*% change from baseline Mean ± SD*	*HPF vs SF*	*% change between HPF vs SF*
VL-MT-7	1.02 ± 0.14	18.56 ± 8.4	1.02 ± 0.19	17.32 ± 8.0	0.971	0.733
VL-CSA-7	2.97 ± 0.35	17.49 ± 6.4	2.72 ± 0.56	21.71 ± 12.5	0.228	0.349
VL-ECH-7	68.2 ± 11.3	36.01 ± 26.4	68.4 ± 11.2	36.11 ± 14.5	0.965	0.992
VL-PA-7	13.3 ± 2.7	34.41 ± 12.5	13.5 ± 2.8	33.22 ± 6.6	0.885	0.785
GS-MT-7	1.58 ± 0.19	13.50 ± 3.0	1.37 ± 0.20	22.84 ± 7.2	0.026	0.001
GS-CSA-7	3.30 ± 0.21	10.86 ± 4.6	3.02 ± 00.52	16.43 ± 5.5	0.118	0.021
GS-ECH-7	54.95 ± 6.3	39.41 ± 13.3	57.78 ± 6.8	42.47 ± 12.2	0.338	0.588
GS-PA-7	16.05 ± 2.4	27.89 ± 10.0	16.64 ± 2.1	26.09 ± 3.8	0.547	0.586

percentage decrease in both muscle thickness and CSA in the VL and gastrocnemius muscles was significantly less pronounced in the HPF group compared to the SF group at days 3, 5, and 7 post-ICU admission ($p < 0.05$).

In ICU patients, most of the changes are estimated by ultrasound were recorded during first day of stay.[2,18] Only a few studies have investigated the effect of protein on muscle wasting. Muscle wasting correlates with physical functional defect and poor survival.[4,19] Ultrasound is a new promising method to identify loss of muscle and has good intra- and inter-observer reliability.[20,21] Several factors leads to loss of muscle like inflammation, immobility, nutrition, and infection.[22] Finally, some studies suggest that also high-dose protein supplement favor muscle protein anabolic effect.[23]

A prospective trial demonstrated that high-dose amino acids showed improved handgrip strength and increased forearm muscle thickness, as measured by ultrasound.[24] Patients who achieved protein and energy targets experienced a decrease in mortality.[25] Another retrospective study involving 23 trauma and septic patients showed that the decrease in protein content was significantly greater in the low-dose protein group than high-dose protein group.[26] These studies showed that optimal protein supplementation decreases the mortality and increases the muscle protein storage. Although we did not measure total protein content, muscle wasting was significantly lower in the high-protein group than the SF patients in the first 7 days of ICU stay.

The existing body of literature includes a limited number of investigations, specifically, two studies, that have examined the impact of high-protein nutritional interventions on skeletal muscle in critically ill patients. In the EAT-ICU trial, enteral feeds were given by using indirect calorimetry and it did not increase the muscle function, as measured by the physical component score (PCS). The muscle functions were measured 6 months after randomized by PCS [which is a sub-score of SF-36 (36-Item Short Form Health Survey questionnaire)].[27] As most muscle changes occur within the first week of critical illness, as described by Puthucheary et al., measuring muscle functions after 6 months will be confounded by multiple factors other than early nutrition (post-recovery nutrition, the incidence of secondary infections, the cumulative nutritional provision over the initial 7 days, relevant laboratory parameters, and the total duration of ICU stay).[2] Here, we used ultrasound to find muscle wasting, which detects earlier than the outcome score used in the study. The whey protein used in our study may have a favorable impact on the muscles as it contains more leucine (17%) compared to standard protein (10%).[28] Leucine is vital for muscle building as the rate of generation of protein is proportional to the leucine content of the meal.[8] In previous work in trauma, patients received either SF or HPF. The study results showed thickness and CSA were decreased in all the patients; however, it was not statistically significant. The present study's design and statistical power were not sufficient to definitively confirm or refute this particular hypothesis.[29] The different patient groups, different muscle groups, and whey protein in the HPF group are significant differences from the previous study. Many patients were in sepsis, in whom the catabolic state and protein homeostasis may be different from that of trauma patients.[16]

Muscle echogenicity can be measured by grayscale analysis with open-source software such as ImageJ (version 1.47, National Institutes of Health, USA). Changes in echogenicity may be due to sarcolemmal edema, fat replacement, and intramuscular fibrosis.[30] These latter findings indicate muscle fiber necrosis and may correlate with impaired muscle function.[31] Loss of echogenicity was associated with poor outcomes like fewer ICU-free days and decreased survival.[17] A recent prospective observational study found that echogenicity increased progressively from day 5 and showed no changes in muscle echogenicity between high and low protein groups in trauma patients.[29] Also, muscle biopsy results indicated that significant muscle necrosis begins after day 7.[2] In our study, we followed only the first week. The previous two studies showed echogenicity, and muscle necrosis was increased significantly day 5 of ICU. Our research has similar results in terms of change in echogenicity with high vs standard protein-based feed. Ultrasound examinations were done only for the first week of ICU admission, as most changes occur during this period.[2] A longer follow-up may be helpful in specific ICU populations to find out any significant change in echogenicity. Other than nutrition, muscle edema and inflammation may also affect echogenicity.

Muscle architecture encompasses various structural characteristics, including the PA, which represents the angle at which muscle fibers insert into the aponeurosis. This angle also provides information about muscle strength. The increased angle of pennation indicates densely packed myofibrils, which in turn results in greater force of contraction.[17] As per our knowledge, no study validated the impact of whey protein nutrition on muscle PA in ICU patients. In this study, a decrease in the PA was observed in both the Standard Formula and High-protein Formula groups during the initial 7 days of ICU admission; however, this change did not reach statistical significance when comparing the two groups. Further

comprehensive research is warranted to elucidate the potential relationship between PA dynamics and protein supplementation strategies in critically ill patients.

Consistent with existing literature, all patients in our cohort exhibited evidence of muscle wasting, as quantified by reductions in muscle thickness and CSA. Clinically, muscle weakness in critically ill patients is frequently symmetric and predominantly affects the proximal musculature of the limbs.[32]

However, muscle wasting predominantly occurred in the lower limb muscles compared to upper limb muscles, which could be explained by disuse atrophy of the leg muscles.[33] Muscle loss is predominantly seen in the rectus femoris compared to the anterior tibialis in trauma patients.[29]

Prior studies have indicated that the magnitude of muscle loss during critical illness may be more pronounced in the gastrocnemius muscle compared to the VL.[33] Our findings align with these observations. This differential susceptibility to atrophy may be attributed to the lower limbs being more prone to disuse in the context of critical illness. More extensive trials are required to analyze quantitative and qualitative muscle changes between different lower limb muscles.

The NUTRIC score identifies patients at risk of malnutrition during critical illness, but the need of diet and weight loss history in this subset of patients is difficult to obtain because most of them are mechanically ventilated.[34] At the time of enrollment in our study, all participants were characterized as having adequate nutritional status (mean NUTRIC = 2.1). No other trials have evaluated the relationship between the NUTRIC score and muscle wasting, and in this study, it was found that there was no significant correlation in both the groups. The PaO_2/FiO_2 ratio usually measures the severity of ARDS. Survivors of ARDS patients had considerable muscle weakness, and it negatively impacted their survival.[35] To the best of our knowledge, this study represents the initial investigation to analyze the correlation between the PaO_2/FiO_2 ratio and muscle wasting on day 7 in both ARDS and non-ARDS patients, revealing no statistically significant association. The SOFA score provides a numerical quantification of the degree of organ dysfunction. Prior research has indicated a direct association between muscle weakness and the severity of organ dysfunction, identifying it as an independent predictor during the first week in ICU patients.[36] However, our analysis did not demonstrate a significant correlation between the baseline SOFA score and the extent of muscle wasting observed in our cohort.

Trauma, COPD, cancer, and neuromuscular disorders are causing significant muscle loss. Quadriceps weakness is seen significantly in COPD patients even in younger age-group, and quadriceps muscle force value decreased with disease severity. As per our knowledge, no study measured the impact of comorbidities on muscle loss in ICU patients other than COPD.[37] In our study, only 12 patients had comorbidities, none with COPD. In sepsis, patients had higher catabolic activity in both respiratory and lower limb muscles of ICU patients, especially in weight-bearing lower limb muscles.[38] Our findings indicate that the gastrocnemius muscle in the Standard Formula group exhibited a significantly greater degree of muscle thickness and CSA wasting compared to the High-Protein Formula group among patients with comorbidities and septic shock. In contrast, these significant differences were not observed in the VL muscle. The precise pathophysiological mechanisms underlying the differential rates of muscle loss in distinct muscle groups remain to be fully elucidated. More extensive studies are needed to validate various comorbidities and septic shock on muscle wasting in ICU patients.

Firstly, to the best of our knowledge, this study represents the first randomized controlled trial specifically designed to compare the effects of high-dose versus standard-dose protein administration on muscle characteristics in critically ill ICU patients. Secondly, this investigation is novel in its prospective evaluation of the impact of nutritional intervention on quantitative (muscle thickness, CSA), qualitative (echogenicity), and architectural (PA) changes in the skeletal muscles of ICU patients utilizing ultrasound imaging. Both groups were well-matched at baseline, with adequate blinding of personnel associated with the trial and the treating team. Follow-up was completed for all patients.

There were certain limitations, such as the small sample size, comparatively less severely ill patients (patients with minimal vasopressor), were included. Further, the impact of inflammatory markers was not noted, which may have some role in muscle wasting. Muscle biopsies could have been better for correlating the qualitative changes found in ultrasound. However, it was not done due to ethical concerns. Although ultrasound is a point-of-care investigation in the ICU and has many advantages in evaluating muscle loss, it has certain limitations when used for musculoskeletal examinations. The measurements are operator-dependent and may have inter- or intra-observer variability. In our study, an average of three readings was considered to avoid any intra-observer variability. Muscle edema has some effect on the muscle thickness and echogenicity measured by ultrasound. An equibalance between intake and output was maintained in our study. However, the impact of muscular edema on echogenicity cannot be ruled out. Another limitation of ultrasound is that there is no standardization of the techniques for different muscles. We followed the technique as described in previous studies, but the limb dominance was not considered.

Conclusion

In intubated ICU patients, high-protein normocaloric (1.5 kg/kg/day of protein and 25 kcal/kg/day calories) nutrition decreases muscle loss in lower limb weight-bearing muscles during the first week of ICU stay. However, the differences in qualitative muscle parameters, like muscle echogenicity and PA, were not significant. Standardization of muscle ultrasound protocols for evaluating muscle loss in ICU patients will help in better quantification of data. A study with a larger number of patients, involving different muscle groups, may provide further information regarding adequate protein supplementation in critically ill mechanically ventilated patients.

Ethics Approval

Institutional ethics committee approval was obtained (NK/1607/MD/10089-90).

Clinical trial registry number: CTRI/2020/02/023512.

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References

1. 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–S308. DOI: 10.1097/CCM.0b013e3181b6ef67.
2. Puthucheary 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. DOI: 10.1001/jama.2013.278481.
3. Moisey LL, Mourtzakis M, Cotton BA, Premji T, Heyland DK, Wade CE, et al. Nutrition and Rehabilitation Investigators Consortium (NUTRIC). Skeletal muscle predicts ventilator-free days, ICU-free days, and mortality in elderly ICU patients. Crit Care 2013;17(5):R206. DOI: 10.1186/cc12901.
4. Herridge MS, Cheung AM, Tansey CM, Matte-Martyn A, Diaz-Granados N, Al-Saidi F, et al. Canadian Critical Care Trials Group. One-year outcomes in survivors of the acute respiratory distress syndrome. N Engl J Med 2003;348(8):683–693. DOI: 10.1056/NEJMoa022450.
5. Kress JP, Hall JB. ICU-acquired weakness and recovery from critical illness. N Engl J Med 2014;370(17):1626–1635. DOI: 10.1056/NEJMra1209390.
6. Wollersheim T, Woehlecke J, Krebs M, Hamati J, Lodka D, Luther-Schroeder A, et al. Dynamics of myosin degradation in intensive care unit-acquired weakness during severe critical illness. Intensive Care Med 2014;40(4):528–538. DOI: 10.1007/s00134-014-3224-9.
7. Fitts RH, Romatowski JG, Peters JR, Paddon-Jones D, Wolfe RR, Ferrando AA. The deleterious effects of bed rest on human skeletal muscle fibers are exacerbated by hypercortisolemia and ameliorated by dietary supplementation. Am J Physiol Cell Physiol 2007;293(1):C313–C320. DOI: 10.1152/ajpcell.00573.2006.
8. Jakubowicz D, Froy O. Biochemical and metabolic mechanisms by which dietary whey protein may combat obesity and type 2 diabetes. J Nutr Biochem 2013;24(1):1–5. DOI: 10.1016/j.jnutbio.2012.07.008.
9. De Jonghe B, Sharshar T, Lefaucheur JP, Authier FJ, Durand-Zaleski I, Boussarsar M, et al. Groupe de Réflexion et d'Etude des Neuromyopathies enRéanimation. Paresis acquired in the intensive care unit: A prospective multicenter study. JAMA 2002;288(22):2859–2867. DOI: 10.1001/jama.288.22.2859.
10. Connolly BA, Jones GD, Curtis AA, Murphy PB, Douiri A, Hopkinson NS, et al. Clinical predictive value of manual muscle strength testing during critical illness: An observational cohort study. Crit Care 2013;17(5):R229. DOI: 10.1186/cc13052.
11. Hough CL. Improving physical function during and after critical care. Curr Opin Crit Care 2013;9(5):488–495. DOI: 10.1097/MCC.0b013e328364d7ef.
12. 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 2015;43(4):897–905. DOI: 10.1097/CCM.0000000000000821.
13. Reimers K, Reimers CD, Wagner S, Paetzke I, Pongratz DE. Skeletal muscle sonography: A correlative study of echogenicity and morphology. J Ultrasound Med 1993;12(2):73–77. DOI: 10.7863/jum.1993.12.2.73.
14. Arts IM, Overeem S, Pillen S, Schelhaas HJ, Zwarts MJ. Muscle ultrasonography to predict survival in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 2011;82(5):552–554. DOI: 10.1136/jnnp.2009.200519.
15. Pillen S, Scholten RR, Zwarts MJ, Verrips A. Quantitative skeletal muscle ultrasonography in children with suspected neuromuscular disease. Muscle Nerve 2003;27(6):699–705. DOI: 10.1002/mus.10385.
16. 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. Crit Care 2013;17(5):R227. DOI: 10.1186/cc13050.
17. Fukunaga T, Kawakami Y, Kuno S, Funato K, Fukashiro S. Muscle architecture and function in humans. J Biomech. 1997;30(5):457–463. DOI: 10.1016/s0021-9290(96)00171-6.
18. Katari Y, Srinivasan R, Arvind P, Hiremathada S. Point-of-care ultrasound to evaluate thickness of rectus femoris, vastus intermedius muscle, and fat as an indicator of muscle and fat wasting in critically ill patients in a multidisciplinary intensive care unit. Indian J Crit Care Med 2018;22(11):781–788. DOI: 10.4103/ijccm.IJCCM_394_18.
19. Saccheri C, Morawiec E, Delemazure J, Mayaux J, Dubé BP, Similowski T, et al. ICU-acquired weakness, diaphragm dysfunction and long-term outcomes of critically ill patients. Ann Intensive Care 2020;10(1):1. DOI: 10.1186/s13613-019-0618-4.
20. Tillquist M, Kutsogiannis DJ, Wischmeyer PE, Kummerlen C, Leung R, Stollery D, et al. Bedside ultrasound is a practical and reliable measurement tool for assessing quadriceps muscle layer thickness. JPEN J Parenter Enteral Nutr 2014;38(7):886–890. DOI: 10.1177/0148607113501327
21. Hadda V, Khilnani GC, Kumar R, Dhunguna A, Mittal S, Khan MA, et al. Intra- and inter-observer reliability of quadriceps muscle thickness measured with bedside ultrasonography by critical care physicians. Indian J Crit Care Med 2017;21(7):448–452. DOI: 10.4103/ijccm.IJCCM_426_16.
22. Fan E, Cheek F, Chlan L, Gosselink R, Hart N, Herridge MS, et al. ATS Committee on ICU-acquired Weakness in Adults; American Thoracic Society. An official American Thoracic Society Clinical Practice guideline: The diagnosis of intensive care unit-acquired weakness in adults. Am J Respir Crit Care Med 2014;190(12):1437–1446. DOI: 10.1164/rccm.201411-2011ST.
23. Hermans G, Casaer MP, Clerckx B, Güiza F, Vanhullebusch T, Derde S, et al. Effect of tolerating macronutrient deficit on the development of intensive-care unit acquired weakness: A subanalysis of the EPaNIC trial. Lancet Respir Med 2013;1(8):621–629. DOI: 10.1016/S2213-2600(13)70183-8.
24. Preiser JC, van Zanten AR, Berger MM, Biolo G, Casaer MP, Doig GS, et al. Metabolic and nutritional support of critically ill patients: Consensus and controversies. Crit Care 2015;19(1):35. DOI: 10.1186/s13054-015-0737-8.
25. Ferrie S, Allman-Farinelli M, Daley M, Smith K. Protein requirements in the critically ill: A randomized controlled trial using parenteral nutrition. JPEN J Parenter Enteral Nutr 2016;40(6):795–805. DOI: 10.1177/0148607115618449.
26. Ishibashi N, Plank LD, Sando K, Hill GL. Optimal protein requirements during the first 2 weeks after the onset of critical illness. Crit Care Med 1998;26(9):1529–1535. DOI: 10.1097/00003246-199809000-00020.
27. Allingstrup MJ, Kondrup J, Wiis J, Claudius C, Pedersen UG, Hein-Rasmussen R, et al. Early goal-directed nutrition versus standard of care in adult intensive care patients: The single-centre, randomised, outcome assessor-blinded EAT-ICU trial. Intensive Care Med 2017;43(11):1637–1647. DOI: 10.1007/s00134-017-4880-3.
28. Farnfield MM, Carey KA, Gran P, Trenerry MK, Cameron-Smith D. Whey protein ingestion activates mTOR-dependent signalling after resistance exercise in young men: A double-blinded randomized controlled trial. Nutrients 2009;1(2):263–275. DOI: 10.3390/nu1020263.
29. Annetta MG, Pittiruti M, Silvestri D, Grieco DL, Maccaglia A, La Torre MF, et al. Ultrasound assessment of rectus femoris and anterior tibialis muscles in young trauma patients. Ann Intensive Care 2017;7(1):104. DOI: 10.1186/s13613-017-0326-x.
30. Kelmenson DA, Quan D, Moss M. What is the diagnostic accuracy of single nerve conduction studies and muscle ultrasound to identify critical illness polyneuromyopathy: A prospective cohort study. Crit Care 2018;22(1):342. DOI: 10.1186/s13054-018-2281-9.
31. Norman H, Zackrisson H, Hedström Y, Andersson P, Nordquist J, Eriksson LI, et al. Myofibrillar protein and gene expression in acute quadriplegic myopathy. J Neurol Sci 2009;285(1–2):28–38. DOI: 10.1016/j.jns.2009.04.041.
32. Henriksson-Larsén KB, Lexell J, Sjöström M. Distribution of different fibre types in human skeletal muscles. I. Method for the preparation and analysis of cross-sections of whole tibialis anterior. Histochem J 1983;15(2):167–178. DOI: 10.1007/BF01042285.
33. Turton P, Hay R, Taylor J, McPhee J, Welters I. Human limb skeletal muscle wasting and architectural remodelling during five to ten days intubation and ventilation in critical care – an observational

study using ultrasound. BMC Anesthesiol 2016;16(1):119. DOI: 10.1186/s12871-016-0269-z.
34. Heyland DK, Dhaliwal R, Jiang X, Day AG. Identifying critically ill patients who benefit the most from nutrition therapy: The development and initial validation of a novel risk assessment tool. Crit Care 2011;15(6):R268. DOI: 10.1186/cc10546.
35. Dinglas VD, Aronson Friedman L, Colantuoni E, Mendez-Tellez PA, Shanholtz CB, Ciesla ND, et al. Muscle weakness and 5-year survival in acute respiratory distress syndrome survivors. Crit Care Med 2017;45(3):446–453. DOI: 10.1097/CCM.0000000000002208.
36. Bednarík J, Vondracek P, Dusek L, Moravcova E, Cundrle I. Risk factors for critical illness polyneuromyopathy. J Neurol 2005;252(3):343–351. DOI: 10.1007/s00415-005-0654-x.
37. Kharbanda S, Ramakrishna A, Krishnan S. Prevalence of quadriceps muscle weakness in patients with COPD and its association with disease severity. Int J Chron Obstruct Pulmon Dis 2015;10:1727–1735. DOI: 10.2147/COPD.S87791.
38. Klaude M, Fredriksson K, Tjäder I, Hammarqvist F, Ahlman B, Rooyackers O, et al. Proteasome proteolytic activity in skeletal muscle is increased in patients with sepsis. Clin Sci (Lond) 2007;12(9):499–506. DOI: 10.1042/CS20060265.