Applied nutritional investigation

Qualitative and quantitative muscle ultrasound changes in patients with COVID-19−related ARDS

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ABSTRACT

Objectives: Severe forms of the novel coronavirus-19 (COVID-19) are associated with systemic inflammation and hypercatabolism. The aims of this study were to compare the time course of the size and quality of both rectus femoris and diaphragm muscles between critically ill, COVID-19 survivors and non-survivors and to explore the correlation between the change in muscles size and quality with the amount of nutritional support delivered and the cumulative fluid balance.
Methods: This was a prospective observational study in the general intensive care unit (ICU) of a tertiary care hospital for COVID-19. The right rectus femoris cross-sectional area and the right diaphragm thickness, as well as their echo densities were assessed within 24 h from ICU admission and on day 7. We recorded anthropometric and biochemical data, respiratory mechanics and gas exchange, daily fluid balance, and the number of calories and proteins administered.
Results: Twenty-eight patients were analyzed (65 ± 10 y of age; 80% men, body mass index 30 ± 7.8 kg/m^2). Rectus femoris and diaphragm sizes were significantly reduced at day 7 (median = −26.1 [interquartile ratio [IQR], = −37.8 to −15.2] and −29.2% [−37.8% to −19.6%], respectively) and this reduction was significantly higher in non-survivors. Both rectus femoris and diaphragm echo density were significantly increased at day 7, with a significantly higher increase in non-survivors. The change in both rectus femoris and diaphragm size at day 7 was related to the cumulative protein deficit ($R = 0.664$, $P < 0.001$ and $R = 0.640$, $P < 0.001$, respectively), whereas the change in rectus femoris and diaphragm echo density was related to the cumulative fluid balance ($R = 0.734$, $P < 0.001$ and $R = 0.646$, $P < 0.001$, respectively).
Conclusions: Early changes in muscle size and quality seem related to the outcome of critically ill COVID-19 patients, and to be influenced by nutritional and fluid management strategies.

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Introduction

Ever since the novel coronavirus disease (COVID-19) was declared a global pandemic, >125 million confirmed cases and >2.5 million confirmed deaths have been recorded globally [1]. Although most cases are characterized by minimal flulike constitutional symptoms or may even be asymptomatic, some patients develop a severe pneumonia that may lead to acute respiratory distress syndrome, multiorgan failure, and death [2].

Systemic hyperinflammation and a procoagulant state play a major pathophysiologic role in these severe forms [3]. The increased glucocorticoid and catecholamine production are the main factors driving hypercatabolism and anabolic resistance [4].

Catabolism from muscle proteins, muscle weakness, and/or atrophy, have all been linked to increased morbidity and mortality [5]. Moreover, patients with inadequate energy and protein intake are at risk for complications and negative outcomes [6]. Adequate reserves of body protein at admission to the intensive care unit (ICU) may then be crucial to recovery and survival, as well as the degree of muscle wasting during the acute phase of the disease.

Even more than other categories of critically ill patients, those admitted to the ICU for severe cases of COVID-19 may accumulate nutritional deficits during their first days of ICU stay, which may then play an important role in ICU and hospital outcomes, including mortality and acquired infections [7]. Recent studies showed that feeding intolerance is common in these patients [8,9], a finding that cannot be solely explained by the deranged gas exchange, the effects of vasoconstriction from vasopressor, or the elevated

doses of sedatives and opioids required to facilitate mechanical ventilation [10].

From a nutritional perspective, it has been shown that reaching both protein and energy targets in critically ill patients had a positive effect on survival [11]. The early caloric deficit has already been showed as an independent predictor of ICU mortality together with Sequential Organ Failure Assessment (SOFA) score, male sex, obesity, and diabetes, suggesting that any effort should be made to implement timely and adequate nutritional support during an ICU stay [12].

Ultrasound is increasingly being used to assess both changes in muscle size and quality over time [13]. Muscle ultrasound has gained wide acceptance as a tool to assess and track changes in muscle structure and composition, potentially improving classification of patients who may be at risk for muscle wasting. Sonography may in fact allow for the assessment of both muscle mass (thickness or cross-sectional area) and quality (echo density) [14]. In particular, it is well known that both diaphragm and limb muscle size, structure, and function deteriorate during the course of an ICU stay; sonographic findings of reduced diaphragm thickness and rectal femoris cross-sectional area (RF CSA) have been found to be associated with poor clinical outcomes [5,15,16]. Similarly, changes in muscle echo density, easily assessed using gray-scale analysis of ultrasound images, have been associated with negative outcomes [17,18].

Few studies have involved investigating, at the same time, the change in respiratory and limb muscle size and quality in critically ill patients [19−21], and none, to the best of our knowledge, did so in relation to the outcome of patients diagnosed with COVID-19−related acute respiratory failure. The main aim of this observational study was to compare the change over the first week in the size and quality of both rectus femoris and diaphragm muscles between critically ill COVID-19 patients who did and did not survive their ICU stay. Secondary outcomes were to explore possible correlations between the change in both muscles size and quality with the amount of nutritional support delivered and the cumulative fluid balance.

Materials and methods

Ethics

Ethical approval for this study was provided by the Comitato Etico Interaziendale Milano Area 1. Written informed consent was obtained according to Italian regulations.

Enrollment criteria

All consecutive patients admitted from November 15 to December 30, 2020, to the general ICU of a tertiary care hospital were considered for enrollment. Inclusion criteria were ≥18 y of age, admission for acute hypoxemic respiratory failure, undergoing invasive mechanical ventilation for ≤48 h and with confirmed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Exclusion criteria were being <18 y; pregnancy; trauma to the right lower limb; history of neurologic, neuromuscular, or muscular wasting disease; and prolonged immobility before admission to the ICU.

Study protocol

Within 24 h after ICU admission, patients underwent diaphragm and rectus femoris ultrasound. The same measurements were repeated at day 7 if the patient was still in the ICU. Anthropometric data, biochemical parameters of inflammation and organ function, respiratory mechanics, and gas exchange data were collected. Fluid balance and the number of calories and proteins administered were recorded on a daily basis.

Management of critically ill patients

All patients were deeply sedated and mechanically ventilated at ICU admission. The clinical management of patients was standardized according to local and regional recommendations [22]. In particular, all patients received muscle relaxants during the first week of ICU stay, as well as a 10-d course of intravenous (IV) dexamethasone 6 mg/d [23]. No other immunomodulatory agents were administered, nor were antiviral drugs.

As far as the nutritional support is concerned, guidelines for management of COVID-19 patients from the European Society for Clinical Nutrition and Metabolism were followed, and hypocaloric nutrition (~70% of estimated energy expenditure) was provided in the early phase of acute illness, with increments up to 80% to 100% between days 3 and 7. As indirect calorimetry was not available, daily energy and protein targets (25 kcal/kg and 1.3 g/kg, respectively) were calculated according to actual body weight (ABW) or adjusted body weight (AjBW), as needed [24]. When no absolute contraindications were present, high-protein enteral feeding (Nutrison Protein Plus, Nutricia International B.V., Hoofddorp, Nederland) was started within 48 h of ICU admission, initially at a rate of 20 mL/h and increased until the desired goal was reached. Enteral feeding was administered via nasogastric tube; a postpyloric access was positioned in case of gastric intolerance despite prokinetic therapy. Calories contained in propofol or glucose solutions, and the use of supplements were accounted for when total calorie and protein delivery were calculated. Adequacy of calories and protein received was expressed as a percentage of the prescribed nutrition.

Data collection

Data on the onset of symptoms, medical history and current medications at time of symptom onset, clinical and laboratory data at admission, treatment data, and outcome were collected. Severity scores (simplified acute severity score [SAPS II] and SOFA) were calculated at admission. The clinical frailty scale was used to summarize the overall level of fitness [25]. Anthropometric measurements of body length and weight were recorded at ICU admission. ABW was obtained by weighing beds when available, or it was defined as the weight reported by the patient immediately before ICU admission, or from information obtained by the families. Ideal body weight (IBW) was defined as the weight based on the patient's height calculated to a body mass index (BMI) of 25 kg/m^2. AjBW, which was used as the reference weight for nutritional targets in obese patients, was calculated as follows:

$$(ABW - IBW) \times 0.33 + IBW \text{ [26]}.$$

Fluid balance was evaluated daily during ICU stay. Fluid intake included IV fluids, total parenteral and enteral nutrition, blood products, and IV medications. Fluid output included urine, feces, blood loss, output from drains and other body cavities, gastric aspirate, and respiratory evaporation [27]. All variables were derived directly from the computerized clinical records (Digistat Intensive Care Unit, Ascom, Baar, Switzerland). The cumulative fluid balance was the sum of the daily balances.

As an index of adequacy of the nutritional support, the cumulative energy and protein deficit were calculated, as the difference between the calorie or protein target and the amount actually delivered to the patient [28].

Ultrasound examination

The right rectus femoris muscle and the right diaphragm were assessed for muscle size and echo density within 24 h after ICU admission and then on day 7. Ultrasound device settings, depth, and gain were kept constant using the same image presets between patients, as previously described [29].

Ultrasound was performed by a single, operator with >10 y of muscle ultrasound experience. B-mode images were obtained utilizing a 6 to 14 MHz linear array on a Esaote MyLab X8 device (Esaote SpA, Genova, Italy) with the patient at 30 degrees for diaphragm measurements [30] and in the supine position for rectus femoris ultrasound [31]. End-expiratory diaphragm thickness was assessed in the zone of apposition of the diaphragm to the rib cage. The linear probe was placed above the right 10th rib in the midaxillary line, as previously described [32] (Fig. 1B). RF CSA was measured with the rested leg supported in passive extension. B-mode ultrasonography using the same linear transducer array was applied, similar to the method previously described [13]. Briefly, the probe was placed three-fifths of the distance from the anterior superior iliac spine to the superior patellar border, transversely to the (Fig. 1A). Because muscle and subcutaneous fat can easily be compressed, a minimal amount of pressure was applied on the tissue under an ultrasound probe sufficiently covered with gel, to optimize imaging conditions. To improve ultrasound reproducibility, we temporarily marked and regularly reinforced the skin landmarks. The cross-sectional area was outlined by a movable cursor on a frozen image and calculated. The methods for image acquisition and analysis of both muscles have previously shown good to excellent reliability [33]. All the measurements are reported as the average of three consecutive measurements within 10%.

Images were saved in JPEG format and echo density was quantified using a gray-scale histogram analysis of the images, with values ranging between 0 (black) and 255 (white). The analysis was performed with ImageJ software (NIH, Bethesda, MD, USA). The hand-free tool was used to define the largest free-form area devoid of artifacts; a gray-scale frequency histogram was then generated for the selected
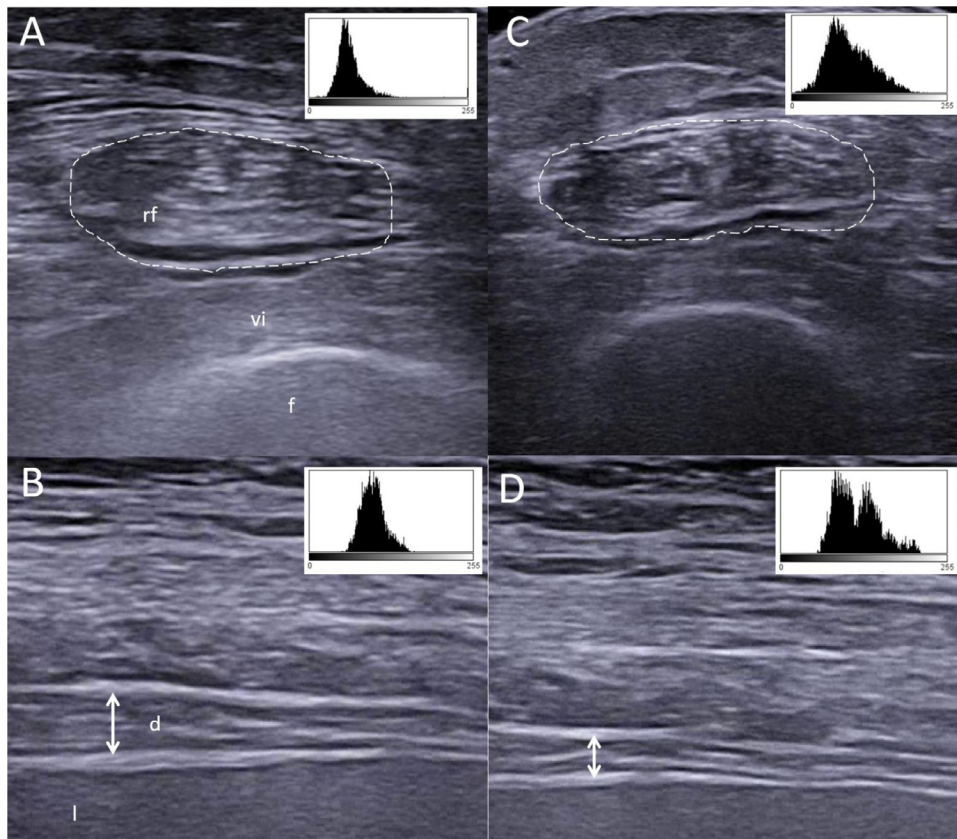
Fig. 1. Muscle ultrasound images at ICU admission (**A**) and on day 7 (**B**) from a representative patient. A and B show the rectus femoris and diaphragm, respectively, at ICU admission in a representative patient. C and D show the same muscles in the same patient on day 7 after ICU admission. The area enclosed in the white dashed line is the rectus femoris cross-sectional area; the arrowhead line indicates the diaphragm thickness. The histogram of muscle echo density is shown on the top right corner of each panel. d, diaphragm; f, femur; l, liver; rf, rectus femoris; vi, vastus intermedius.

region, and the median value was recorded [34]. The assumption is that the higher the average density of a muscle region of interest, the lower is its quality (i.e., more intramuscular fat, edema, or connective tissue). Images were reviewed by a second investigator who was not directly involved in image acquisition. Figure 1 shows the muscle ultrasound images at ICU admission and on day 7 from a representative patient.

Reproducibility was assessed by building a Bland−Altman plot and calculating the intraclass correlation coefficient (ICC) [35]: Admission images were assessed a second time by the same investigator for intraobserver reproducibility, whereas day 7 images were analyzed by a second, independent investigator for interobserver reproducibility.

Statistical analysis

Given the lack of similar investigations in COVID-19 patients, a priori sample size calculation was not performed; the sample size was pragmatically based on a 2-mo time frame as well as recently published literature on similar topics [17,18]. Patients with only one ultrasound measurement (i.e., those discharged alive or who died before day 7) were excluded from the analysis.

We compared the variables in patients who did and did not survive the ICU stay. Comparisons between normally distributed variables were performed by Student's *t* test, whereas non-normally distributed variables were compared by Wilcoxon signed-rank test. Normality was tested by the Shapiro−Wilk test. Normally distributed data are indicated as mean ± SD, while median and interquartile range (IQR) were used to report non-normally distributed variables. Association between two variables was assessed by linear regression. The comparison between survivors and non-survivors was performed by analysis of variance for repeated measurements, with time as a within-subject factor and the outcome as a fixed, between-subject factor. The model included the interaction effect of time on the outcome. The statistical significance of the within-subject factor was corrected with the Greenhouse-Geisser method. Pairwise, post hoc multiple comparisons were carried out according to Tukey method.

The statistical analysis was carried out with STATA version 14 (StataCorp, College Station, TX, USA); two-tailed $P < 0.05$ were considered for statistical significance.

Results

Thirty-six consecutive patients were enrolled in the study. Eight patients died before day 7; ultrasound images at admission and after 7 d were available for the remaining 28 patients. Supplementary Figure 1 shows the diagram of patient flow during the study period.

Table 1 shows the demographic data, severity scores at admission, comorbidities, main treatments received during ICU stay that could have affected muscle mass, and outcomes of patients who did and did not survive. Mean age was 65 ± 10 y, about 80% were men, and the main comorbidities were hypertension and obesity. The onset of symptoms before hospital admission was on average 5 d (IQR= 3; 7), whereas intubation was performed 9 d (IQR = 7; 15) after symptom onset. Overall ICU mortality was 58.3%.

Supplementary Table 1 shows the biochemistry, mechanical ventilation, and gas exchange at ICU admission and at day 7 in ICU survivors and non-survivors. Inflammatory markers and parameters of organ function did not differ between the groups at ICU admission. Similarly, there were no statistically significant differences in the setting of positive end-expiratory pressure and fraction of inspired oxygen (FiO_2), or gas exchange and respiratory mechanics between patients who did and did not survive. Similar findings were observed at day 7, with the exception of a significantly lower oxygenation (partial pressure of oxygen/FiO_2 85 [IQR = 61−103] versus 98 [IQR = 84−131], $P = 0.0407$) and arterial pH (7.37 [IQR = 7.35−7.41] versus 7.42 [IQR = 7.41−7.46], $P = 0.0211$) in patients who went on to die.

Table 1
Demographic data, severity scores at admission, comorbidities, and outcomes of survivors and non-survivors

Variable	Survivors (n = 14)	Non-survivors (n = 14)	P-value
Anthropometric measures			
Male sex, n (%)	11 (78.6)	11 (78.6)	>0.999
Age (y), mean ± SD	62 ± 10	67 ± 10	0.1537
Height (cm), mean ± SD	169 ± 5	171 ± 9	0.6424
Actual body weight (kg)	90 [75−95]	80 [71−91]	0.3685
BMI (kg/m²), mean ± SD	30.2 ± 6.2	29.9 ± 8.9	0.8774
Ideal body weight (kg), mean ± SD	64 ± 4	65 ± 5	0.5429
Severity scores			
SAPS II score	31 [25−37]	37 [30−41]	0.0579
SOFA score	3 [3−4]	4 [3−6]	0.5391
Clinical Frailty scale	2 [1−3]	2 [2−3]	0.1439
Charleson comorbidity index	3 [3−5]	5 [3−6]	0.0711
Comorbidities, n (%)			
Hypertension	6 (42.8)	8 (57.1)	0.450
Obesity	7 (50)	5 (35.7)	0.445
Diabetes	4 (28.6)	5 (35.7)	0.686
COPD	0	1 (7.1)	0.309
Treatment before ICU admission			
Duration of symptoms before hospitalization (d)	5 [3−7]	5 [2−9]	0.7411
CPAP before ICU admission, n (%)	14 (100)	13 (92.9)	0.973
Duration of symptoms before CPAP (d)	6 [3−7]	5 [2−8]	0.9745
Duration of symptoms before intubation (d)	9 [7−14]	9 [5−16]	0.9170
Treatment during ICU stay			
Average plasma glucose (mg/dL), mean ± SD	148 ± 17	149 ± 14	0.9344
Use of aminoglycosides	0	0	>0.999
Use of norepinephrine, n (%)	6 (42.8)	4 (28.6)	0.430
Norepinephrine average dose (mcg·kg·min⁻¹), mean ± SD	0.11 ± 0.05	0.12 ± 0.06	0.8631
Use of muscle relaxants, n (%)	14 (100)	13 (92.9)	0.309
Atracurium average dose (mg·kg·h⁻¹), mean ± SD	0.7 ± 0.1	0.7 ± 0.1	0.8404
Use of systemic corticosteroids, n (%)	11 (78.6)	12 (85.7)	0.622
Outcomes			
Duration of mechanical ventilation (d)	14 [9−21]	16 [7−24]	0.8871
ICU LOS (d)	19 [14−26]	16 [7−24]	0.2415
Ventilator-free days (d)	2 [1−4]	0 [0−0]	<0.0001
Hospital LOS (days)	47 [30−57]	21 [15−28]	0.0052
ICU, n (%)	0 [0]	14 [100]	–
Patients who developed VAP, n (%)	10 (71.4)	14 [100]	0.0311
Patients who developed bacteremia, n (%)	9 (24.3)	12 (85.7)	0.1900

BMI, body mass index; COPD, chronic obstructive pulmonary disease; CPAP, continuous positive airway pressure; ICU, intensive care unit; LOS, length of stay; SAPS II, Simplified Acute Physiology Score, second version; SOFA, Sequential Organ Failure Assessment score; VAP, ventilator-associated pneumonia.
All values median [IQR] unless otherwise noted.

Figure 2 shows the time course of energy and protein intake during the first 7 d of ICU stay in survivors and non-survivors. Energy intake significantly increased from admission to day 7 ($P < 0.0001$), with no statistically significant differences between the groups ($P = 0.1482$). On the other end, protein intake, which also significantly increased over time, was higher in survivors ($P < 0.0001$ for both factors). Calorie intake from propofol ranged from 25.6% to 33.3% of total daily calories in survivors and from 24.6% to 40% in non-survivors ($P = 0.4550$).

Muscle ultrasound was performed in all patients. Figure 3 shows the evolution of muscle mass (RF CSA and diaphragm thickness) and echo density over time. The ICC for intrarater reproducibility were RF CSA 0.997 (IQR = 0.994−0.998), diaphragm thickness 0.992 (IQR = 0.984−0.996], rectus femoris echogenicity 0.997 (IQR = 0.995−0.998), and diaphragm echogenicity 0.998 (IQR = 0.996−0.999); the values for interrater reproducibility were RF CSA 0.994 (IQR = 0.987−0.997), diaphragm thickness 0.982 (IQR = 0.962−0.992), rectus femoris echogenicity 0.998 (IQR = 0.997−0.999) and diaphragm echogenicity 0.998 (IQR = 0.997−0.999). Supplementary Figures 2 through 5 show the agreement between the raters for the different measurements.

Table 2 shows the size and echo density of rectus femoris and diaphragm muscles at ICU admission and on day 7 in survivors and non-survivors. A significant reduction in the RF CSA was recorded after the first 7 d of ICU stay. Moreover, the cross-sectional area was lower in non-survivors. Similar findings were seen for the end-expiratory diaphragm thickness. Both rectus femoris and diaphragm echo density significantly increased during the first 7 d of ICU stay, and in both cases non-survivors had a significantly higher echo density.

Table 3 shows the nutritional parameters on day 7 as well as the changes in muscle ultrasound size and echo density from admission to day 7 in survivors and non-survivors. No differences were found in urine nitrogen output or serum prealbumin levels. Moreover, the cumulative energy deficit over the first week of ICU stay did not differ between the groups. On the other hand, the cumulative protein deficit was significantly higher in ICU non-survivors, as was the cumulative fluid balance. The reduction in both rectus femoris and diaphragm size from baseline to day 7 was significantly greater in non-survivors, as was the increase in echo density.

The change in both RF CSA and diaphragm end-expiratory thickness form baseline to day 7 was inversely related to the cumulative protein deficit ($R^2 = 0.441$, $P < 0.001$ and $R^2 = 0.409$, $P < 0.001$, respectively), and was not related to the cumulative fluid balance ($R^2 = 0.146$, $P = 0.054$ and $R^2 = 0.020$, $P = 0.409$, respectively). The change in rectus femoris and diaphragm echo density was positively related to the cumulative fluid balance ($R^2 = 0.539$, $P < 0.001$ and $R^2 = 0.417$, $P < 0.001$, respectively), and it was not related to the cumulative protein deficit ($R^2 = 0.126$, $P = 0.064$ and $R^2 = 0.083$, $P = 0.137$, respectively; Fig. 4).

Discussion

To the best of our knowledge, this was the first study to investigate the time course of both respiratory and limb muscle mass and quality in critically ill patients with COVID-19, and to relate the findings with the nutritional strategy and the outcomes. The main findings of this prospective, observational study were as follows:

- Both RF CSA area and diaphragm thickness were significantly reduced after 1 wk of ICU stay and this phenomenon was significantly higher in ICU non-survivors;
- Both rectus femoris and diaphragm echo density were significantly increased after the first week of ICU stay with a significantly higher increase in ICU non-survivors;
- The decrease in both diaphragm and rectus femoris size was significantly related to the protein deficit over the first week; and
- The increase in both diaphragm and rectus femoris echo density was significantly related to the cumulative fluid balance over the first week of ICU stay.

One of the aims of nutritional support is to potentially mitigate the loss of lean tissue during a state of hypermetabolism and catabolism and consequently improve patient outcomes. Significant muscle wasting occurs early in critically ill patients [36]. The underlying mechanisms, despite still being under investigation, are likely multifactorial and include altered substrate metabolism, anabolic resistance, hypoxia, inflammation, immobilization, and nutritional inadequacy [37]. Notably, all of the former have been described in severe cases of COVID-19. A recent study with
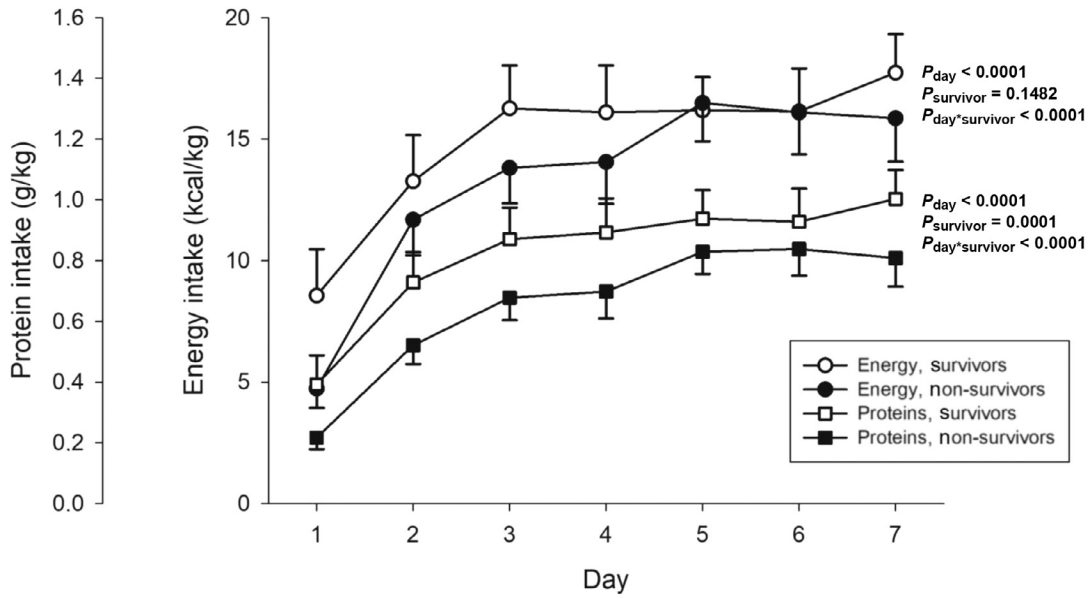
Fig. 2. Time course of energy and protein intake during the first 7 d of stay in ICU survivors and non-survivors. White circles represent energy intake in ICU survivors, black circles represent energy intake in non-survivors; white squares represent protein intake in ICU survivors, and black squares represent protein intake in ICU non-survivors. The analysis was performed with factorial analysis of variance, see the methods section for further details. ICU, intensive care unit.

critically ill patients with COVID-19−related acute respiratory failure showed that even achieving the supposed nutrition targets, the nitrogen balance remained negative by, on average, −9 g/d

over the first week of stay [4]. Moreover, a recent prospective investigation showed that only about 70% of critically ill COVID-19 patients reached their caloric target on day 4, whereas <25%
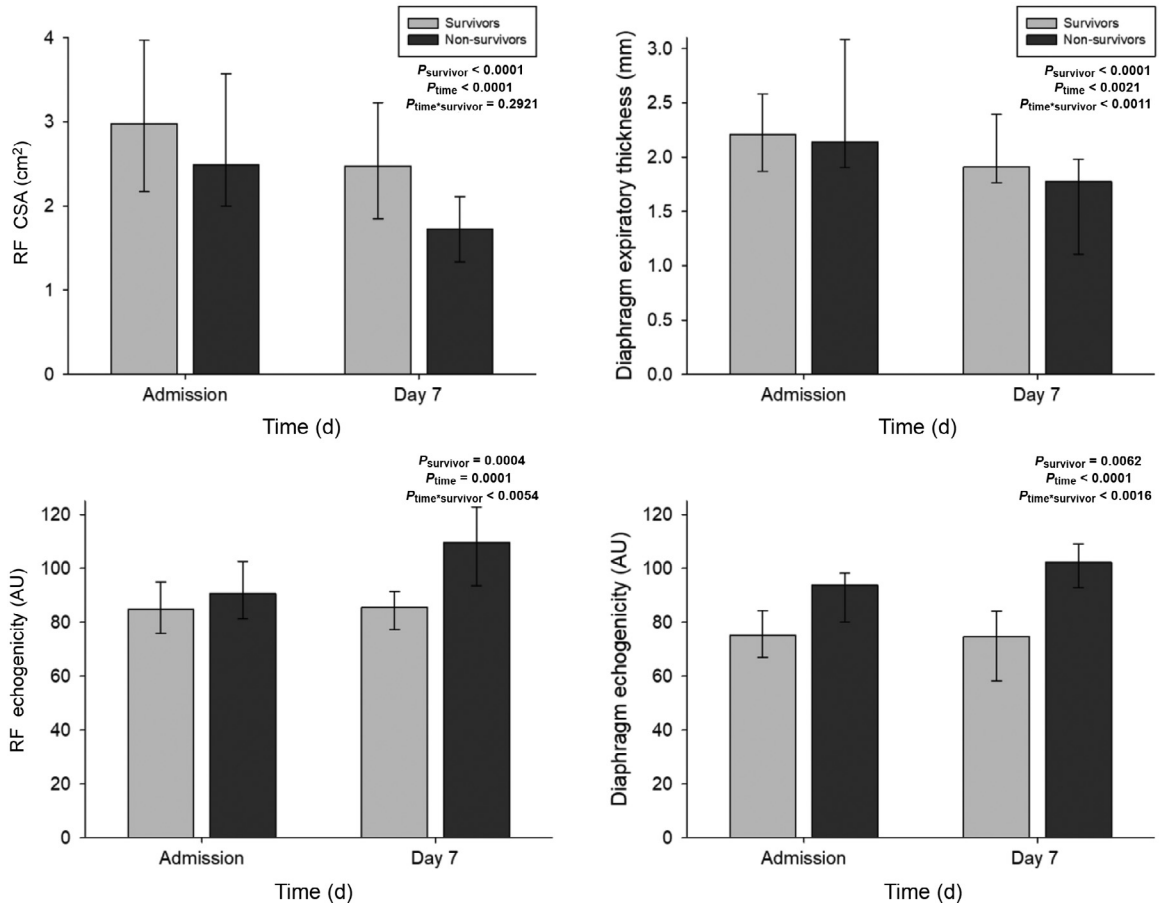

Fig. 3. Evolution of muscle mass (RF CSA and diaphragm thickness) and echo density over time. The figure shows the median (box), first and third quartile of each variable. Data were analyzed with two-way analysis of variance, see the methods section for further details. AU, arbitrary unit; RF CSA, rectus femoris cross-sectional area.

Table 2
Size and echo density of rectus femoris and diaphragm muscles in ICU survivors and non-survivors at admission and on day 7

	Survivors (n = 14)	Non-survivors (n = 14)	$P_{outcome}$	P_{time}	$P_{time \times outcome}$
RF CSA (cm^2)			<0.0001	<0.0001	0.2911
Admission	2.98 [2.17−3.97]	2.49 [2.04−3.34]			
Day 7	2.48 [1.85−3.23]	1.73 [1.38−2.09]			
Rectus femoris echodensity (AU)			0.0004	<0.0001	0.0054
Admission	84.9 [75.9−94.9]	90.6 [82.9−102.2]			
Day 7	85.5 [77.4−91.4]	109.6 [93.6−121.1]			
Diaphragm thickness (mm)			<0.0001	0.0021	0.0011
Admission	2.21 [1.87−2.58]	2.14 [1.95−3.00]			
Day 7	1.91 [1.80−2.35]	1.78 [1.11−1.98]			
Diaphragm echo density (AU)			0.0062	<0.0001	0.0016
Admission	75.1 [66.8−84.4]	93.7 [82.2−97.9]			
Day 7	74.7 [58.4−83.7]	102.3 [93.1−108.9]			

AU, arbitrary unit; ICU, intensive care unit; RF CSA, rectus femoris cross-sectional area.
All data median [IQR].

reached their protein target by the same day [38]. A recent longitudinal investigation showed that severe and critical COVID-19 patients showed a 30% reduction in RF CSA with an average 16.8% increase in echo-density from days 1 to 10 [39]. We confirmed an early and rapid wasting of both respiratory and limb muscles in patients with COVID-19−related acute respiratory failure, which was even more pronounced in those who eventually died. Moreover, despite a protocolized nutritional strategy, calorie intake did not differ among groups (likely because of the significant amount of energy provided as propofol infusion), whereas the nutritional intake of proteins was lower in non-survivors. Nitrogen output on day 7 suggested a similar catabolism in the two groups, which may reflect the contribution of an unintended lower protein intake in non-survivors. Indeed, the observational nature of the present study precluded any inference about the causal nature of this association.

In humans, COVID-19 causes anorexia, myalgias, and muscle loss. SARS-CoV-2 spike protein uses the angiotensin-converting enzyme 2 (ACE2) receptor to bind to and subsequently enter the cells. Skeletal muscle cells express an ACE2 receptor, which might in part explain the muscle symptoms. This, combined with immobility and invasive mechanical ventilation, can lead to a severe form of sarcopenia during the acute phase of COVID-19 [40,41]. Indeed, diaphragm and limb muscles are known to be different in their composition, as different as their susceptibility to a given injury [42]. We chose to focus on the rectus femoris because it was shown that lower (but not upper) limb muscle thickness and architecture undergo early rapid changes after ICU admission, potentially reducing force generation and contributing to ICU-acquired

weakness [43]. Skeletal muscle injury was also reported to be directly associated with SARS-COV-2 infection [44].

As far as the diaphragm is concerned, a wide array of factors, including mechanical ventilation and suppression of spontaneous inspiratory effort [15,45,46], were shown to lead to an early and acute diaphragm injury in critically ill patients. Indeed, in patients with severe forms of COVID-19 undergoing invasive mechanical ventilation, a large and prolonged use of muscle relaxants has been described [47]. We speculated that patients with COVID-19−related acute respiratory failure might be at significant risk for diaphragm and limb muscle wasting, and we sought to assess the association of such wasting with the nutritional strategy and the outcome. To do so, we used quantitative muscle ultrasound, which is a non-invasive, reproducible, and relatively inexpensive imaging modality that facilitates the assessment of muscle mass and quality based on tissue composition [13,14]. We found that a significant reduction in muscle mass and change in muscle structure occurred in all patients; such changes were more pronounced in non-survivors and were significantly related to the cumulative protein deficit and the fluid balance.

Despite the sample being composed of relatively young men with few comorbidities and in an overall "fit" state as assessed by the low comorbidity index and clinical frailty scale, the results of the present study confirmed the rapid and significant deterioration in skeletal muscle size and quality compared with previous studies in unselected ICU patient groups [5,17,36,48]. We observed an average decrease in RF CSA and diaphragm thickness of 27% and 29%, respectively, with even higher decreases in non-survivors. Quite interestingly, we were unable to find any difference between

Table 3
Nutritional parameters and change in muscle ultrasound size and echo density on day 7 in ICU survivors and non-survivors

|	Survivors (n = 14)	Non-survivors (n = 14)	P-value
Urine nitrogen output (g/d)	38.2 [27.3−52.4]	34.4 [24.2−42.8]	0.3853
Cumulative fluid balance (mL)	2090 [−376 to 4365]	3821 [3175−6070]	0.0356
Cumulative protein deficit (g)	249 [199−361]	382 [307−476]	0.0052
Cumulative energy deficit (kcal)	4145 [2800−4900]	4475 [4150−5926]	0.1145
Prealbumin (g/100 mL)	18.5 [16−24]	13.5 [9−17]	0.1372
Triacylglycerols (mg/100 mL)	276 [203−352]	227 [172−276]	0.2170
Change in rectus femoris cross-sectional area (%)	−17.9 [−22.9 to −12.0]	−36.3 [−42.4 to −28.7]	0.0021
Change in rectus femoris echo density (%)	7.2 [−8.3 to 18.9]	16.7 [9.3−29.9]	0.0387
Change in diaphragm thickness (%)	−19.9 [−25.7 to −2.7]	−37.8; [−43.1 to −29.4]	0.0003
Change in diaphragm echo density (%)	0.1 [−12.6 to 17.5]	14.6 [9.5−24.3]	0.0169

All values median [IQR].
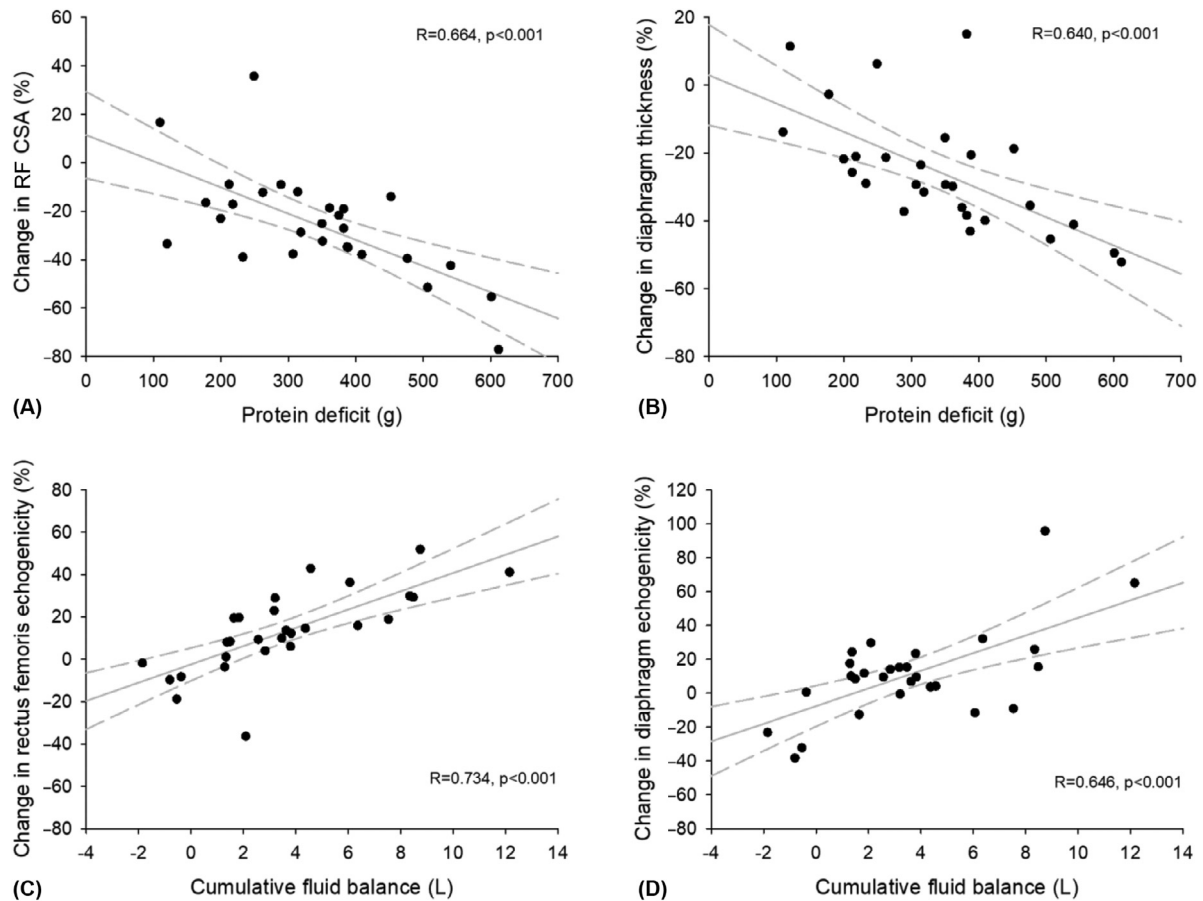
Fig. 4. Correlation between the change in muscle size over the first week and the cumulative protein deficit (A and B), and the change in muscle echo density over the first week and the cumulative fluid balance. RF CSA, rectus femoris cross-sectional area.

the groups in the other factors that are known to effect muscle mass during ICU stay, such as glycemic control, vasoactive drugs, and aminoglycoside antibiotics. In other studies, even smaller changes were associated with physical function at ICU discharge [49], in-hospital mortality, length of mechanical ventilation, and development of ICU-acquired weakness [50]. Several previous studies have shown decreases of diaphragm thickness during the early course of mechanical ventilation, associated with an impairment in diaphragm function and a poor clinical outcome [51]. At least some mechanically ventilated patients experience early rapid increases in diaphragm thickness, which predicted prolonged ventilation, raising the possibility of a clinically significant diaphragm injury caused by insufficient respiratory muscle unloading during ventilation [15]. In the present investigation, we were unable to find any patient with increased baseline diaphragm thickness, likely because all of the patients were undergoing controlled mechanical ventilation and receiving muscle-relaxant agents, then excluding the chance of overuse myotrauma.

Consistent with previous reports, non-survivors had at admission a lower size and a higher density in both the diaphragm and the rectus femoris. Previous investigations showed that both a low skeletal muscle area or density, as assessed by computed tomography (CT) scan, upon ICU admission are associated with an increased mortality, independent of the severity of disease [52,53]. In the present study, muscle mass and quality was assessed using ultrasound; indeed, rectus femoris echo density, as a marker of muscle quality, increased over the first week by on average 12%, which is similar to previously published data [16,17,31,48,49].

More recently, changes in diaphragm echo density during the early course of mechanical ventilation were described and associated with a negative outcome [18]. In both cases, the change increments in muscle echo density are presumed to be the consequence of either inflammation or infection, or edema due to fluid shifts in the context of volume resuscitation with a positive fluid balance [54]. Interestingly, we found that diaphragm echo density did not change from baseline in patients who eventually went on to survive, which could, in theory, suggest a recovery of an initial early muscle injury. As a matter of fact, due to the stress imposed on the health care system by the wave of the big surge in the number of COVID-19 patients, several patients underwent prolonged non-invasive ventilator assistance while waiting for an available ICU bed, and we cannot exclude the development of under assistance myotrauma from insufficient ventilator support before intubation [55].

Several studies found associations between protein intake and improved outcomes, at least in specific subgroups of patients such as those at high nutritional risk or with normal kidney function [56–58]. We found an association between a negative protein deficit and a larger reduction in muscle mass, which may potentially be the link between protein administration and improved outcomes. Indeed, an early higher protein intake was associated with lower mortality in patients with low skeletal muscle area and density but not in patients with normal skeletal muscle area [59]. A low muscle area (i.e., a low muscle mass) is considered a proxy for low protein reserves, whereas low muscle density is associated with qualitative changes such as muscle inflammation and fatty

infiltration, which may in turn create an environment of low-grade inflammation and insulin resistance, thereby contributing to anabolic resistance. Identifying these patients might improve risk stratification and help guide treatments; however, CT scan assessment of muscle mass and quality is limited by its logistic and health-related risks. We have shown that the ultrasound evaluation of muscle mass and quality might provide clinically meaningful information, with the certain advantages of a bedside-available, repeatable, safe, low-cost procedure.

Notably, and similar to previous investigations [53,60,61], ICU non-survivors had a significantly higher positive cumulative fluid balance compared with survivors, as well as a cumulative negative protein deficit. In an effort to investigate the causes of such difference, we measured the nitrogen output on day 7, which did not statistically differ between the groups. From this, we inferred that the reason for a protein deficit was not the increased loss but a reduced intake. In fact, the amount of protein administered with the nutritional support was significantly lower in non-survivors (Fig. 2). One possible explanation for a reduced protein administration might be a higher incidence of intolerance to enteral feeding in non-survivors, which is known to be a marker of a more severe disease state and associated with a worse outcome, especially in the COVID-19 population [62]. No differences were found in the cumulative energy (calorie) deficit, likely because a significant part of the calorie provision came from IV lipids from propofol infusion. Similar findings were recently reported in a population of surgical critically ill patients [28]. The rationale behind the negative effects of a cumulative protein deficit may depend on a decreased immunocompetence, an increased skeletal muscle catabolism, and impaired wound healing. As a matter of fact, several recent investigations support the increasingly appreciated importance of protein in improving survival [53,56]. The exact amount of protein requirement in critical illness remains controversial, especially because this is generally scaled on body weight instead of a more individualized approach [53]. Further studies will elucidate whether the use of muscle ultrasound might play a role in the optimization and individualization of nutrient delivery and potentially providing a tool to evaluate the effect of different nutrition regimens.

As for the association between a positive cumulative fluid balance and a worse outcome, this has been known for quite a long time in the general ICU population and in septic patients [63,64]. Unfortunately, we did not collect enough data to explain the possible reasons for a positive cumulative fluid balance in non-survivors, although this might depend on the higher severity of non-survivors (higher SAPS II score at admission, higher ferritin and interleukin-6), which might have caused a greater hemodynamic affect and prompted a more aggressive fluid resuscitation.

As we did not measure diaphragm or limb muscle function, the precise pathophysiologic meaning of the changes in size and echo density and the potential mechanistic basis for their association with a negative outcome are unraveled and deserve further investigation. A strong correlation between increased echo density and inflammation was confirmed in muscle biopsy studies [16,65]. On the other hand, it is possible that changes in echo density may depend on the accumulation fluid resuscitation-related tissue edema [16]. At variance with previous studies [18], we found that changes in echo density of both muscles were significantly related to a positive fluid balance. This may reflect the different patient population and the peculiar characteristics of COVID-19 patients, in whom endothelial activation and dysfunction play a key role in the pathogenesis of the disease, by altering the integrity of vessel barrier and potentially favoring fluid extravasation.

Limitations

As a major limitation to this study, we first recognize the use of predictive equation in the estimation of energy needs, and the lack of daily nitrogen balance calculations, hence protein deficit was used instead of the actual balance. Indeed, this approach has already been used and resulted in clinically meaningful association with patient outcomes [28]. Second, given the short-term of the inclusion period (first week of ICU stay), no volitional or long-term measures were assessed. Moreover, we did not record structural aspects of the muscle such as the pennation angle or fascicle length, which may be associated with the force-generating capacity of the muscle [43]. Third, measurements of diaphragm thickness can be difficult in some individuals as a poor acoustic window occurs in <5% of ICU patients [66], and even less when the right hemidiaphragm is imaged. Moreover, it is known that adiposity might have a negative effect on the quality of ultrasound imaging [67], and the average BMI of the population we enrolled indicated that about half of the patients could be defined as obese. However, in the present investigation, the right hemidiaphragm could be assessed by ultrasound and images were recorded and analyzed in all the patients. Fourth, given the limited dimension of the diaphragm (generally around 2 mm), results of the investigation of the diaphragm might be underpowered as compared with those for the rectus femoris. Finally, the observational nature and limited sample size of the present study do not allow for any inference about causality to be made: The results are hypothesis-generating only, and the association a higher protein deficit, a reduced muscle mass, and a higher mortality might be confounded by less severely ill patients reaching higher protein intakes.

Conclusions

The present data suggest that early changes in muscle size and quality may potentially be related to the outcome and be influenced by nutritional and fluid management strategies. As we await further, larger investigations to confirm our findings, we hypothesize that using muscle ultrasound for early identification of patients with or at risk for muscle wasting may help to promote individualized nutritional interventions or earlier allocation/enhanced intensity of physical rehabilitation, with the aim of preserving respiratory and limb muscle size and architecture.

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