Ultrasound assessment of rectus femoris and anterior tibialis muscles in young trauma patients

Maria Giuseppina Annetta1, Mauro Pittiruti2, Davide Silvestri1, Domenico Luca Grieco1*, Alessio Maccaglia1, Michele Fabio La Torre3, Nicola Magarelli3, Giovanna Mercurio1, Anselmo Caricato1 and Massimo Antonelli1

Abstract

Purpose: Quantitative and qualitative changes of skeletal muscle are typical and early findings in trauma patients, being possibly associated with functional impairment. Early assessment of muscle changes—as evaluated by muscle ultrasonography—could yield important information about patient’s outcome.

Methods: In this prospective observational study, we used ultrasonography to evaluate the morphological changes of rectus femoris (RF) and anterior tibialis (AT) muscles in a group of young, previously healthy trauma patients on enteral feeding.

Results: We studied 38 severely injured patients (median Injury Severity Score = 34; median age = 40 y.o.) over the course of the ICU stay up to 3 weeks after trauma. We found a progressive loss of muscle mass from day 0 to day 20, that was more relevant for the RF (45%) than for the AT (22%); this was accompanied by an increase in echogenicity (up to 2.5 by the Heckmatt Scale, where normal echogenicity = 1), which is an indicator of myofibers depletion.

Conclusions: Ultrasound evaluation of skeletal muscles is inexpensive, noninvasive, simple and easily repeatable. By this method, we were able to quantify the morphological changes of skeletal muscle in trauma patients. Further studies may rely on this technique to evaluate the impact of different therapeutic strategies on muscle wasting.

Keywords: Muscle mass, Muscle ultrasonography, Enteral feeding, Trauma

Background

Muscle wasting is a frequent finding in critically ill patients and is associated with worse short- and long-term outcomes. Loss of mass and function of skeletal muscles starts early—in the first 24 h after admission to intensive care unit (ICU)—and may persist for years (‘post-ICU syndrome’). Loss of muscle mass is a major cause of ICU-acquired muscle weakness and is associated with delayed weaning, prolonged ICU and hospital stay and is an independent predictor of 1-year mortality [1–3]. Long-term muscle impairment may be responsible of physical, mental and cognitive dysfunction, which affects the quality of life of ICU survivors and increases the costs of the healthy care services [4–6]. Early physical rehabilitation has been associated with conflicting results in terms of functional outcome [7, 8], so that the best strategy would theoretically be to avoid or minimize muscle loss during ICU stay, for example delivering an appropriate nutritional support. Unfortunately, limited data clarify the possible impact of adequate calories and protein delivery on skeletal muscle preservation and long-term outcome of muscular function [9–11]; also, the conclusions of the few available clinical studies are controversial. Some studies have even suggested that increasing protein intake in the early phase of critical illness may accelerate muscle loss during the first week [4, 12].

The sequential assessment of quantitative and qualitative changes of muscle mass may help identify critically ill patients with high risk of muscle dysfunction, as well...
as verify the effects of different nutritional regimens. In this regard, B-mode ultrasonographic evaluation of skeletal muscles (in particular, rectus femoris and anterior tibialis) is an emerging and reliable tool to assess muscle changes over time. It is a bedside technique, easy to use and inexpensive [13]. Its cost-effectiveness is higher than CT scan evaluation, which has been used for the same purpose [2].

In this prospective clinical study, we evaluated the feasibility of detecting the quantitative and qualitative changes of rectus femoris and anterior tibialis muscles over the course of the ICU stay up to 20 days from admission in a cohort of young trauma patients.

**Methods**

This was a prospective observational study performed in a cohort of severe multiple trauma patients admitted to the 20-bed ICU of our institution (Fondazione Poli clinico ‘A.Gemelli’—University Hospital) in a period of 10 consecutive months. All subjects were on enteral feeding.

**Patients**

We enrolled exclusively young trauma patients with an injury severity score (ISS) exceeding 25, who were admitted to our ICU within few hours after the injury. We recruited only well-nourished, previously healthy subjects, aged 18–59 y.o, with no past history of nutritional problems, chronic use of drugs nor orthopedic issues (such as skeletal fractures or immobilization) in the previous 2 years. Trauma patients not fulfilling this criteria were not considered for the enrollment. Exclusion criteria were: relevant comorbidities (renal, liver or heart disease or COPD), previous immune abnormalities (including treatment with corticosteroids), neuromuscular disease, past or recent history of cancer.

For each patient, demographics and clinical data were recorded: age, height, weight, body mass index (BMI), ISS, APACHE II score, Sequential Organ Failure Assessment score (SOFA), Glasgow Coma Scale (GCS)—both total and motor (GCS-M)—Glasgow Outcome Scale (GOS), number of days on mechanical ventilation, ICU length of stay, incidence of secondary infections, daily provision of calories and protein, blood levels of albumin, total protein and creatinine, blood urea nitrogen (BUN) and other laboratory data. Among mechanically ventilated patients, weaning was classified as simple, difficult or prolonged, as previously described [14]. Infections were classified according to definitions by the Center for Disease Control and Prevention [15].

**Nutritional support**

Enteral feeding was started as soon as the patient was hemodynamically stable and fully resuscitated, usually within 24 h. Our nutritional target was to achieve a minimal protein intake of 0.8 g/kg/day within day 5 after admission. Patients who did not reach this target for any reason (gastrointestinal intolerance or contraindication to enteral feeding or repeated forced suspensions of enteral feeding because of multiple surgical procedures) were excluded from the final analysis. We used either a standard feeding formula (1.5 total kcal/ml, protein 60 g/L) or a high-protein formula (1.35 total kcal/ml, protein 75 g/L): These different regimens were not assigned by randomization, but they were the result of a change of nutritional policies in our ICU during the period of the study; though, the two groups of patients—nourished by a standard formula (SF) or nourished by a high-protein formula (HPF)—were similar in terms of age, Injury Severity Score (ISS), Acute Physiology and Chronic Health Evaluation (APACHE II score), height and weight. Tube feeding was started at a very low rate (10–20 ml/h) and increased at each 24-h interval as tolerated, in order to meet a minimal protein intake of 0.8 g/kg/day. The patient’s intolerance to feeding was defined on the basis of clinical signs (abdominal distention, vomiting, increase in serum lactate, high gastric residual volume).

**Ultrasoundography of skeletal muscles**

An ultrasound (US) evaluation of the rectus femoris (RF) and anterior tibialis (AT) muscles was performed in all patients at day 0 (within 24 h from trauma), 5, 10, 15 and 20. We used an US device with a 5- to 7.5-MHz linear probe (Esaote MyLab). According to a previously described methodology [13, 16], skeletal muscles were evaluated by US scan, collecting both quantitative and qualitative data. The transducer was placed perpendicular to the long axis of the muscle (i.e., perpendicular to the major axis of the limb), at 3/5 of the distance between the anterior superior iliac spine and the superior border of patella (i.e., about 15 cm from the patella) for the RF and 5 cm below the peroneal head for the AT muscle. The measurement points were marked with indelible ink to ensure day-to-day consistency and facilitate subsequent measurements. Visualization was consistently obtained, while the patients were supine with both legs in passive extension. Two measurements were taken for each muscle in each leg. In the presence of lower limb fractures, measurements were taken on the contralateral leg only. Excess contact gel was applied so to minimize underlying soft tissue distortion. One operator only (specifically trained in muscle ultrasonography) performed all measurements. US settings (depth, gain and focus) were standardized for both RF and AT examinations. After freezing the US image, quantitative parameters were recorded for both muscles: anterior–posterior diameter (AP diam); lateral–lateral diameter (LL diam); and cross-sectional...
and all of them had a good nutritional status on admis-
sion (median BMI = 25). All patients were severely
injured (ISS = 34, APACHE II score = 16), and most of
them had associated brain injury (84%). There were no
significant differences between patients on SF versus
HPF.

Muscular changes are shown in Table 2.

Primary endpoints of our study were the qualitative and
quantitative changes of skeletal muscles during 3 weeks
of ICU stay, taking into consideration the role of protein
intake. The study protocol was approved by the Ethics
Committee of our institution (Prot. 10917/15).

Statistical analysis
Qualitative data are expressed as number of events (%) and
continuous data as median [interquartile range]. Differ-
ence in the distribution of qualitative variables in SF versus
HPF group was investigated with the Chi-square test or Fisher’s exact test, as appropriate. Difference in the
distribution of quantitative and ordinal variables in SF versus HPF group was assessed with the Mann–Whitney test. In the overall population, the significance of changes in the quantitative variables over time was determined with the one-way ANOVA test for repeated measures; paired comparisons between 2 consecutive
timepoints were then analyzed with the Wilcoxon sum of ranks test. The effect of SF versus HPF on the change in the quantitative variables over time was determined with the two-way ANOVA test for repeated measures; paired comparisons between the distribution of quantitative variable in SF versus HPF group at each time point were also assessed with the Mann–Whitney test.

The entire analysis was conducted applying a bilateral
null hypothesis; accordingly, results with two-tailed
*p < 0.05 were considered significant.

Statistical analysis was conducted with SPSS 20.0.

Results
A total of 120 trauma patients admitted to our ICU were
screened for possible inclusion in our prospective study, and only 52 met the requirements. Nine patients did not
reach the minimal protein intake by enteral feeding. Of
the remaining patients, five died during the first 3 weeks.
The final analysis was conducted on 38 patients, whose characteristics are listed in Table 1. All patients were
young (median age 40 year old); most were male (76%),
and all of them had a good nutritional status on admis-
sion (median BMI = 25). All patients were severely
injured (ISS = 34, APACHE II score = 16), and most of
them had associated brain injury (84%). There were no
significant differences between patients on SF versus
HPF.

Nutritional intake is shown in Table 3. We had 20
patients in the SF and 18 in the HPF group. Mean protein
intake after day 5 was of 0.87 g/kg/day in the SF group
and 1.6 g/kg/day in the HPF group. Mean total calories
were 19 kcal/kg in the SF group and 30 kcal/kg in the HPF

group. No major differences were detected in the main
labaratory values (Table 4), though HPF patients had
nonsignificantly higher blood protein levels (< 0.07)
and significantly higher albumin levels (< 0.03) at day
20.

Discussion
In our study, we adopted ultrasonography for the evalu-
ation of quantitative and qualitative changes of skel-
etal muscles in a homogeneous group of young trauma
patients who were previous healthy, well nourished and
physically active. A previous similar study published
few years ago [4] was focused on a heterogeneous group of critically ill patients with only 25% of them being trauma victims. All previous clinical studies with muscle ultrasonography have been conducted in mixed ICU populations that included medical and surgical, as well as acute and chronic, critically ill patients [4, 13, 19]. On the contrary, in our study there were no confounding factors such as old age, comorbidities, cancer and long-term use of medications.

Quantitative changes of skeletal muscles
In ICU patients, the daily amount of muscle loss—as estimated by US—is reported to range between 6 [20] and 12.5% between day 1 and 7 [4]. Muscle wasting correlates with the ICU length of stay [16] and can be predictive of long-term functional disability [21]. Several factors contribute to muscle wasting over the course of the critical illness, both in the acute and chronic phase: inflammation, neuroendocrine stress response, immobilization, impaired microcirculation and denervation (in the acute phase); infections, nutritional deficiency, hyperglycemia, drugs [22] (in the late phase). Other predisposing factors are: age, baseline muscle function, nutritional status, comorbidities (COPD, renal and heart disease, cancer) [22, 23]. Finally, some data suggest that also parenteral nutrition may worsen muscle function [24].

A retrospective study demonstrated a correlation between hospital mortality and skeletal muscle mass, as estimated by abdominal CT scan [2]: this is particularly evident in trauma patients [25], but apparently not in ICU patients with acute lung injury [26].

Ultrasound has been used to rate the loss of skeletal muscles in patients with orthopedic trauma, COPD, cancer and neuromuscular disorders [13, 27, 28]; indeed, it appears as an emerging field of interest in ICU. Ultrasoundography is more accurate than anthropometric

## Table 1 Demographics

|                      | ALL   | SF n 20 | HPF n 18 |
|----------------------|-------|--------|---------|
| Female sex, n (%)    | 9 (24)| 5 (25) | 4 (22)  |
| Age (years)          | 40 [31–54] | 47 [37–58] | 37 [29–46] |
| Height (cm)          | 175 [167–180] | 175 [168–180] | 170 [167–180] |
| Weight (k)           | 75 [70–81] | 80 [70–90] | 75 [67–85] |
| BMI                  | 25 [23–28] | 26 [23–29] | 23 [24–26] |
| GCS at inclusion     | 7 [3–9] | 7 [3–9] | 6 [3–10] |
| GCS-M at inclusion   | 4 [1–5] | 4 [1–5] | 4 [1–5] |
| Injury Severity Score (ISS) | 34 [27–42] | 34 [28–40] | 34 [27–45] |
| Apache II score      | 16 [13–20] | 16 [13–19] | 18 [12–22] |
| SOFA                 | 7 [5–9] | 8 [5–10] | 6 [5–9] |
| Brain injury n (%)   | 32 (84) | 17 (85) | 15 (83) |
| Thoracic trauma n (%)| 30 (79) | 16 (80) | 14 (78) |
| Abdominal trauma n (%)| 17 (45) | 9 (45) | 8 (44) |
| Pelvic trauma n (%)  | 15 (40) | 8 (40) | 7 (40) |
| Spinal trauma n (%)  | 21 (55) | 10 (50) | 11 (61) |
| GOS 28 days          | 3 [3, 4] | 3 [3, 4] | 3 [3, 4] |
| GCS-M at discharge   | 6 [4–6] | 6 [5, 6] | 6 [4–6] |
| Tracheostomy n (%)   | 24 (63) | 12 (60) | 12 (67) |
| Weaning              |       |        |         |
| Simple, n (%)        | 21 (55) | 10 (50) | 11 (61) |
| Difficult, n (%)     | 11 (29) | 6 (30) | 5 (28) |
| Prolonged, n (%)     | 6 (16) | 4 (20) | 2 (11) |
| Days of mechanical ventilation | 13 [11–19] | 14 [9–22] | 13 [11–17] |
| Patients with at least one documented infection, n (%) | 34 (90) | 19 (95) | 15 (83) |
| Patients with at least one documented infection MDR infection, n (%) | 23 (61) | 13 (65) | 10 (56) |
| Patients with septic shock during the ICU stay, n (%) | 7 (18) | 5 (25) | 2 (11) |
| ICU length of stay   | 22 (17–33) | 22 (16–37) | 22 (17–31) |
| ICU outcome, died, n (%) | 3 (8) | 2 (10) | 1 (6) |

Data expressed as median [interquartile range], if not otherwise specified.
See abbreviations in the text.
measurements and has been shown to closely correlate with the data obtained by MRI and CT scan [19, 29], with the advantage of being less expensive, less time-consuming and safer, since it does not imply radiation exposure. Though some studies have shown a good intra-rater and inter-rater reliability for US measurement of muscle CSA or thickness in adult critically ill patients [4, 30], the matter is still somehow controversial [31, 32].

All of our trauma patients (100%) experienced severe muscle mass loss, as estimated by CSA. Almost half (45%) of RF muscle mass was lost by day 20 with the greatest reduction (21%) occurring after day 15. In a previous work [4], a 17.7% reduction in RF cross-sectional area was shown in a group of mixed ICU patients from day 1 to day 10, with the major loss occurring during the first 7 days. We found a less important reduction in AT cross-sectional area (22%) by day 20.

The exact underlying mechanisms of dissimilar magnitudes of losses in different muscle groups are still unknown. In both rodent and human models, the rate and magnitude of muscle loss seem to depend on both muscle type and degree of inactivity [33, 34]. In experimental and clinical models of lower limb immobilization, muscle loss is greater in the extensor muscles (soleus and gastrocnemius). This is consistent with the greater muscle loss we report in RF (extensor muscle) as compared

| Table 2: Muscle ultrasound |
|---------------------------|
|                           | Day 0     | Day 5     | Day 10    | Day 15    | Day 20    |
| RF: AP diam (mm)          |           |           |           |           |           |
| All pts                   | 17 [15–20]| 17 [15–20]| 16 [13–19]| 14 [12–17]| 13 [9–15]|
| SF                        | 17 [15–20]| 18 [16–20]| 19 [14–20]| 15 [12–17]| 14 [9–17]|
| HPF                       | 17 [15–19]| 16 [15–20]| 16 [12–18]| 13 [12–17]| 9 [9–12]  |
| RF: LL diam (mm)          |           |           |           |           |           |
| All pts                   | 43 [40–46]| 41 [38–44]| 40 [37–45]| 40 [36–44]| 38 [34–41]|
| SF                        | 41 [40–44]| 41 [37–42]| 39 [35–42]| 40 [34–44]| 35 [34–40]|
| HPF                       | 43 [40–47]| 44 [37–46]| 44 [37–47]| 40 [37–47]| 41 [37–43]|
| RF: CSA (cm²)             |           |           |           |           |           |
| All pts                   | 6.1 [5.1–7.3]| 5.9 [4.8–6.3]| 5.1 [4.3–6.2]| 4.6 [3.8–5.3]| 3.5 [3.2–4.7]|
| SF                        | 6.1 [5.1–7.3]| 5.9 [4.9–6.3]| 5.6 [4.2–6.2]| 4.7 [3.9–5.4]| 3.5 [3.2–4.8]|
| HPF                       | 6.3 [4.7–7.3]| 5.9 [4.3–6.5]| 5 [4.2–6.6]| 4.4 [3.5–5.1]| 3.5 [2.8–4.4]|
| RF: echogenicity, Heckmatt Scale |
| All pts                   | 1 [1, 2]| 2 [1, 2]| 2 [1–2.5]| 2 [1–3]| 2.5 [1.5–3]|
| SF                        | 1 [1, 2]| 2 [1, 2]| 2 [1–3.8]| 2.3 [1.6–3.5]| 2.5 [2, 3]|
| HPF                       | 1.3 [1, 2]| 1.5 [1, 2]| 1 [1, 2]| 1.5 [1–2.5]| 2.5 [1–3]|
| AT: AP diam (mm)          |           |           |           |           |           |
| All pts                   | 22 [20–25]| 21 [19–23]| 20 [17–22]| 19 [17–22]| 18 [16–20]|
| SF                        | 22 [20–24]| 21 [19–24]| 20 [18–23]| 20 [17–23]| 19 [15–21]|
| HPF                       | 24 [20–26]| 22 [19–23]| 21 [17–22]| 18 [17–20]| 18 [18]|
| AT: LL diam (mm)          |           |           |           |           |           |
| All pts                   | 25 [22–26]| 24 [21–26]| 23 [21–25]| 22 [20–25]| 22 [20–27]|
| SF                        | 25 [23–27]| 24 [21–27]| 23 [21–26]| 23 [19–26]| 25 [21–28]|
| HPF                       | 23 [22–26]| 24 [21–26]| 22 [21–25]| 21 [20–24]| 22 [20–22]|
| AT: CSA (cm²)             |           |           |           |           |           |
| All pts                   | 5.6 [4.5–6.4]| 4.8 [3.7–5.6]| 4 [3.7–5.2]| 4 [3.3–4.8]| 4.2 [3.4–4.7]|
| SF                        | 5.7 [4.7–6.8]| 4 [3.6–6]| 3.9 [3.7–5.2]| 4 [3.3–5.7]| 4 [3.2–5.2]|
| HPF                       | 5.5 [4.5–6.2]| 4.9 [4.2–5.9]| 4.2 [3.6–5.3]| 3.9 [3.3–4.5]| 4.3 [3.4–4.6]|
| AT: echogenicity, Heckmatt Scale |
| All pts                   | 2 [1, 2]| 2 [1–3]| 2 [1–3]| 1.5 [1–3]| 2.5 [1–4]|
| SF                        | 1.8 [1–2.1]| 2.3 [1.3–3]| 2 [1–3]| 2 [1–3.4]| 3.5 [1–4]|
| HPF                       | 2 [1, 2]| 2 [1–3]| 1.3 [1–3]| 1.3 [1–2.5]| 1.5 [1–3]|

Data expressed as median [interquartile range]

See abbreviations in the text

See statistical significance in the text
Also, RF is a power muscle made up predominantly of type II fast-twitch fibers, while AT muscle composition is mainly made of type I slow-twitch fibers [35]. The preferential loss of a certain kind of muscle fibers might be a crucial determinant of long-term outcome, especially in the development of ICU-acquired weakness and in success of physical rehabilitation. Muscle weakness is usually symmetric and predominates in the proximal part of the limbs (shoulders and ankles) [36]. Laboratory model of ischemic injury has shown that muscle with predominance of fast-twitch fibers had significantly greater necrosis than those richer in slow-twitch fibers [37]. Immobilization studies have shown a preferential loss of type II fibers and conversion of fiber typing from type I to type II in postural muscles [38].

### Qualitative changes of skeletal muscles

Several studies have demonstrated that pathological muscle changes (such as fatty infiltration, atrophy and intramuscular fibrosis) can be detected by ultrasound. Alteration of muscle echogenicity may be ascribed to muscle edema (in the early phase) but also to fibrosis and fatty degeneration (in the late phase). These latter

### Table 3  Nutritional intake

|                  | Day 0–5   | Day 5–10  | Day 10–15 | Day 15–20 | SF versus HPF p value |
|------------------|-----------|-----------|-----------|-----------|-----------------------|
| Enteral nutrition (ml/day) |           |           |           |           | 0.007                 |
| SF               | 850 [625–900] | 1000 [1000–1400] | 1050 [950–1500] | 1350 [1000–1500] |                       |
| HPF              | 1000 [700–1200] | 1500 [1175–1500] | 1500 [1500–1700] | 1500 [1500–2000] |                       |
| Proteins (g/day) |           |           |           |           | 0.001                 |
| SF               | 47 [32–63]  | 67 [50–91] | 70 [48–90] | 70 [50–90] |                       |
| HPF              | 74 [40–93]  | 112 [99–136] | 136 [112–136] | 120 [106–136] |                       |
| Total calories (kcal/day) |           |           |           |           | 0.005                 |
| SF               | 1059 [763–1200] | 1500 [1160–1915] | 1500 [1050–2062] | 1500 [1250–2200] |                       |
| HPF              | 1326 [934–1564] | 1958 [1762–2250] | 2250 [1920–2341] | 2250 [1920–2560] |                       |

**Data expressed as median [interquartile range]**

The overall p value refers to the comparison between the whole time series of changes SF versus HPF

* p < 0.05 for the comparison of SF versus HPF at each time point

See abbreviations in the text

### Table 4 Laboratory data

|                | Day 0      | Day 5      | Day 10     | Day 15     | Day 20     | SF versus HPF p value |
|----------------|------------|------------|------------|------------|------------|-----------------------|
| **BUN (mg/dl)** |            |            |            |            |            | 0.51                  |
| SF             | 12 [10–15] | 17 [13–26] | 20 [17–30] | 25 [17–35] | 24 [18–47] |                       |
| HPF            | 13 [11–17] | 18 [12–22] | 23 [19–29] | 20 [16–24] | 30 [23–37] |                       |
| **Creatinine (mg/dl)** |        |            |            |            |            | 0.46                  |
| SF             | 0.8 [0.7–1] | 0.6 [0.5–0.8] | 0.6 [0.5–0.8] | 0.6 [0.5–0.7] | 0.6 [0.5–1.6] |                       |
| HPF            | 1 [0.6–1.2] | 0.7 [0.5–0.8] | 0.6 [0.5–0.7] | 0.6 [0.4–0.7] | 0.7 [0.5–0.8] |                       |
| **Phosphate (mg/dl)** |         |            |            |            |            | 0.41                  |
| SF             | 2.7 [2–3.5] | 2.9 [2.3–3.4] | 3 [2.5–3.3] | 3.5 [2.7–3.8] | 3.5 [2.8–4.1] |                       |
| HPF            | 3.1 [2.5–4] | 2.9 [2.1–3.6] | 3.5 [2.5–4] | 3.4 [2.6–4.1] | 3.6 [3.3–4] |                       |
| **Proteins (g/L)** |           |            |            |            |            | 0.07                  |
| SF             | 4.7 [3.9–6.1] | 5.2 [4.7–5.6] | 5.8 [5.2–6.3] | 6.1 [5.3–6.5] | 5.8 [5.2–6.4] |                       |
| HPF            | 5.3 [5–5.7] | 5.4 [5, 6] | 5.8 [5.2–6.6] | 6.4 [5.7–7.3] | 7.3 [5.6–8.3] |                       |
| **Albumin (g/L)** |           |            |            |            |            | 0.03                  |
| SF             | 2.9 [2.4–3.4] | 2.6 [2.2–2.9] | 2.6 [2.1–2.9] | 2.8 [2.3–3] | 2.5 [2.1–2.9] |                       |
| HPF            | 3.3 [3–3.6] | 2.7 [2.5–3.1] | 2.7 [2.3–3.1] | 3 [2.8–3.3] | 3.1 [2.6–3.7] |                       |

**Data expressed as median [interquartile range]**

The overall p value refers to the comparison between the whole time series of changes SF versus HPF

* p < 0.05 for the comparison of SF versus HPF at each time point

See abbreviations in the text
findings may be an indicator of quantitative loss of muscular myofibers and disruption of muscle architecture and may correlate with impaired muscle function [18]. Since edema cannot alter the bone signal in contrast to fibrous tissue, changes in muscle echogenicity are related to the fibrous tissue content and with specific structural damage in muscle architecture as seen with muscle biopsies [4] or with muscle magnetic resonance imaging [18]. Structural muscle changes detected by the increased echogenicity have been correlated with measures of muscle strength and function [39].

In our study, echogenicity was quantified by the Heckmatt Scale (Table 5), previously used in the critically ill setting by Grimm [18]. A higher grade of echogenicity with reduced bone signal correlates with the severity of myopathy [17]. Of course, the use of a semiquantitative method such as the Heckmatt Scale may be biased by observer dependency and technical misinterpretation in contrast to objective, user-independent algorithms for image analysis such as computer-assisted quantitative grayscale analysis [40]. Though, the Heckmatt Scale has the relevant advantage of being a rapid and inexpensive bedside technique that can be easily used in the intensive care setting.

Changes in muscle architecture have been documented in previous studies on ICU patients and are associated with increased length of stay in ICU [4, 16, 18, 27, 29]. Changes in muscle echogenicity [4, 18] suggest an alteration of myofibers content, secondary to edema from capillary leak or inflammation. These data are confirmed by muscle biopsies on day 1 and 7 after ICU admission, showing muscle necrosis and macrophage infiltrate [4].

In our study, we found a progressive increase in both RF and AT echogenicity from day 5 on. An alteration of echogenicity was already evident in AT muscle soon after admission and tended to increase in the following weeks. The early alteration of echogenicity in AT but not in RF may have many explanations; probably, post-traumatic edema was more pronounced in the muscles of the lateral/posterior part of the limb (AT) than in the anterior area (RF).

### Table 5 Heckmatt Scale: visual grading scale to classify muscle echo intensity (Ref 17)

| Grade | Ultrasound appearance                      |
|-------|-------------------------------------------|
| Grade 1 | Normal                                   |
| Grade 2 | Increased muscle echo intensity with distinct bone echo |
| Grade 3 | Marked increased muscle echo intensity with a reduced bone echo |
| Grade 4 | Very strong muscle echo and complete loss of bone echo |

### Muscle wasting and nutrition
An optimal provision of energy and protein has been regarded as an important factor improving the patient’s chance of survival and satisfactory clinical outcome [41]. Provision of an optimal amount of protein has been shown to improve the rate of protein synthesis in tissues with rapid turnover, though it did not reduce the catabolic response to injury [42, 43]. In one recent multicenter study [44], provision of at least 80% of the prescribed protein (i.e., 1 g/kg/day) reduced mortality in a ICU population. In patients on parenteral nutrition, protein delivery may be more important than caloric support in terms of short-term outcome [11].

In our study, we found no difference in muscle mass loss or in muscle echogenicity between patients fed with standard (SF) versus high-protein formulas (HPF): though, no conclusion can be drawn in this regard, since the study was not designed or powered to verify such hypothesis. Nonetheless, this finding may be consistent with previous studies showing that depletion of lean body mass, particularly skeletal muscle, is not influenced by nutritional support [45–47] and with studies showing an inverse correlation between the amount of protein delivery and the cross-sectional area of RF [4].

Immobility and inflammation—rather than inadequate nutritional support—might be major determinants of loss of muscle. Inactivity is a potent stimulus to muscle protein breakdown and activation of the ubiquitin–proteasome pathway of proteolysis [48]. Immobility of limbs is quite common in ICU patients and is related to bed rest and sedation. Acute and chronic activation of inflammatory pathway is another potent stimulus for proteolysis [49]. Though, in our study we did not measure any index of inflammatory activity and we could not verify such contention.

### Conclusions
In conclusion, we found that ultrasonography was an easy, effective and practical tool for the daily estimate of changes in skeletal muscles and we confirmed the feasibility of such methodology in trauma patients. Our data show that early loss of muscle mass is particularly relevant also in young trauma patients and that extensor muscles such as rectus femoris are much more affected than flexor muscles (anterior tibialis). Such quantitative muscle loss is associated with an increased echogenicity, possibly associated with progressively impaired muscle function.

### Abbreviations
AP diam: anterior–posterior diameter; APACHE: acute physiologic and chronic health evaluation; AT: anterior tibialis; BMI: body mass index; BUN: blood urea nitrogen; COPD: chronic obstructive pulmonary diseases; CSA: cross-sectional
area; CT: computerized tomography; GCS: Glasgow Coma Scale; GCS-M: Glasgow Coma Scale (motor); GOS: Glasgow Outcome Scale; HPF: high-protein feeding formula; ICU: intensive care unit; ISS: Injury Severity Score; LL diam: latero-lateral diameter; MDR: multiple drug resistant; RF: rectus femoris; SF: standard feeding formula; SOFA: sequential organ failure assessment; US: ultrasound.

Authors’ contributions
MGA, MP and MA designed the study and drafted the manuscript. DLG, DS, MFLT and NV participated in the acquisition of data. AM, AC and GM participated in the data analysis. All authors edited the manuscript and approved the final manuscript. All authors read and approved the final manuscript.

Author details
1 Department of Anesthesia and Intensive Care, Fondazione Policlinico Universitario ‘A.Gemelli’, Largo A.Gemelli, 8, 00168 Rome, Italy. 2 Department of Surgery, Fondazione Policlinico Universitario A.Gemelli, Rome, Italy. 3 Department of Radiology, Fondazione Policlinico Universitario ‘A.Gemelli’, Rome, Italy.

Acknowledgements
None.

Competing interests
The authors declare that they have no competing interests and that they have full control of all primary data.

Availability of data and materials
The authors agree to allow the journal to review their data if requested.

Consent for publication
Not applicable.

Ethical approval and consent to participate
The study protocol was approved by the Ethics Committee of our Hospital (Prot. 10917/15). Written consent was obtained by the patients or by the closest relatives, as required by our Ethics Committee.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 3 February 2017 Accepted: 30 September 2017
Published online: 06 October 2017

References
1. De Jonghe B, Bastuji-Garin S, Durand MC, Malissin I, Rodriguez P, Cerf C, et al. Respiratory weakness is associated with limb weakness and delayed weaning in critical illness. Crit Care Med. 2007;35:2007–15.
2. Wejs PM, Loosjaard WGPMA, Dekker IM, Stapel SN, Girbes AR, et al. Low skeletal muscle area is a risk factor for mortality in mechanically ventilated critically ill patients. Crit Care. 2014;18:R12.
3. Herrmans G, Van Mechelen H, Clerckx B, Vanhullebusch T, Mesotten D, et al. Acute outcome and 1-year mortality of ICU-acquired weakness: a cohort study and propensity matched analysis. Am J Respir Crit Care Med. 2014;190:410–20.
4. Pathucheze YA, Rawal J, McPhail M, Connolly B, Ratnayake G, et al. Acute skeletal muscle wasting in critical illness. JAMA. 2013;310(15):1591–600.
5. Fan E, Dowdy DW, Colantuoni E, Mendez-Tellez PA, Sevriansky JE, Shanholz C, et al. Physical complications in acute lung injury survivors: a 2-year longitudinal prospective study. Crit Care Med. 2013;42:849–59.
6. Herridge MS, Tansey CM, Mattei A, Tomlinson G, Diaz-Granados N, Cooper A, et al. Functional disability 5 years after acute respiratory distress syndrome. N Engl J Med. 2011;364:1293–304.
7. Schweickert WD, Pohlman MC, Pohlman AS, Nigos C, Pavlik AJ, Esbrook CL, et al. Early physical and occupational therapy in mechanically ventilated, critically ill patients: a randomized controlled trial. Lancet. 2009;373:1874–82.
8. Denehy L, Skinner EH, Edbrooke L, Haines K, Warren S, Haworth G, et al. Exercise rehabilitation for patients with critical illness: a randomized controlled trial with 12 months follow-up. Crit Care. 2013;17:R156.
9. Alberda C, Gramlich L, Jones N, Jeejeebhoy K, Day AG, Dhaliwal R, Heyland DK. The relationship between nutritional intake and clinical outcome in critically ill patients: results of an international observational study. Intensive Care Med. 2009;35:1728–37.
10. Wejs PJ, Stapel SN, de Groot SD, Driessen RH, de Jong E, Girbes AR, et al. Optimal protein and energy nutrition decreases mortality in mechanically ventilated, critically ill patients: a prospective observational cohort study. JPEN J Parenter Enteral Nutr. 2012;36:60–8.
11. Ferre 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.
12. Cesaer MP, Wyller A, Hermans G, Wouters PJ, Mesotten D, Van den Bergh E. Role of disease and macronutrient dose in the randomized controlled EPIaNIC trial. a post hoc analysis. Am J Respir Crit Care Med. 2013;187:247–55.
13. Seymour JM, Ward K, Sidhu PS, Puthucheary Z, Steijer J, et al. Ultrasound measurement of rectus femoris cross-sectional area and the relationship with quadriiceps strength in COPD. Thorax. 2009;64:418–23.
14. Boles JM, Bion J, Connors A, Herridge M, Marsh B, Melot C, et al. Weaning from mechanical ventilation. Eur Respir J. 2007;29:1033–56.
15. Horan TC, Andrus MA, Dudeck MA. CDC/NHSN surveillance definition of health-care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control. 2008;36:309–32.
16. Grutther W, Benesch T, Zorn C, Paternostro-Sluga T, Quittan M, Falka-Moser V, et al. Muscle wasting in intensive care patients: ultrasound observation of the quadriceps femoris muscle layer. J Rehabil Med. 2008;40:185–9.
17. Heckmatt JZ, Pier N, Dubowitz V. Real-time ultrasound imaging of the muscle. Muscle Nerve. 1988;11:56–65.
18. Grimm A, Teschner U, Porzelius C, Ludewig K, Zielke J, et al. Muscle ultrasonography for early assessment of critical illness neuromyopathy in severe sepsis. Crit Care. 2013;17:R227.
19. Parsi MT, Mourtzakis M, Day A, Leung R, Watharkar S, Koazar R, et al. Validation of bedside ultrasound of muscle layer thickness of the quadriiceps in the critically ill patient (VALIDUM study): a prospective multicenter study. JPEN J Parenter Enteral Nutr. 2017;41(2):171–80.
20. Campbell IT, Watt T, Withers D, England R, Sukumar S, Keegan MA, et al. Muscle thickness, measured with ultrasound, may be an indicator of lean tissue wasting in multiple organ failure in presence of edema. Am J Clin Nutr. 1995;62:533–9.
21. dos Santos C, Hussain SNA, Marthur S, Picard M, Herridge M, Correa J, et al. Mechanism of chronic muscle wasting and dysfunction after an intensive care unit stay: a pilot study. Am J Respir Crit Care Med. 2016;194(7):821–30.
22. Fan E, Cheek F, Chlan L, Gosselink R, Hart N, Herridge MS, et al. 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;190:1437–46.
23. Farhan H, Moreno-Duarte I, Latronico N, Zafonte R, Eikermann M. Acquired muscle weakness in the surgical intensive care unit: Nosology, epidemiology, and prevention. Anesthesiol. 2016;124:207–34.
24. Hermans G, Cesaer MP, Clerckx B, Gusa 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;1:621–9.
25. Moisey LL, Mourtzakis M, Cotton BA, Premi T, Heyland DK, Wade CE, et al. Skeletal muscle predict ventilator-free days, ICU-free days and mortality in elderly ICU patients. Crit Care. 2013;17:R206.
26. Sheean PM, Peterson SJ, Gomez Perez S, Troy KL, Patel A, Sclamberg JS, et al. The prevalence of sarcopenia in patients with respiratory failure classified as normally nourished using computed tomography and subjective global assessment. JPEN J Parenter Enteral Nutr. 2014;38:873–9.
27. Campbell SE, Adler R, Sofka CM. Ultrasound of muscle abnormalities. Ultrason Q. 2005;21:87–94.
28. Pillem S, Zwartz MJ. Muscle ultrasound in neuromuscular disorders. Muscle Nerve. 2008;37:679–93.
29. Reeves ND, Maganaris CN, Narici MV. Ultrasonographic assessment of human skeletal muscle size. Eur J Appl Physiol. 2004;91:116–8.
30. Tillquist M, Kutsogiannis DJ, Wischmeyer PE, Kummerlen K, Leung R, 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):886–90.

31. Segers J, Hermans G, Charususin N, Fizev T, Vanhorebeek I, et al. Assessment of quadriceps muscle mass with ultrasound in critically ill patients: intra- and inter-observer agreement and sensitivity. Intensive Care Med. 2015;41(3):562–3.

32. Fizev T, Hendrickx A, Van Herpe T, Vlasselaers D, Desmet L, et al. Analysis of reliability and accuracy of muscle thickness ultrasonography in critically ill children and adults. JPEN J Parenter Enteral Nutr. 2016;40:944–9.

33. Zhong H, Roy RR, Siengthai B, Edgerton VR. Effects of inactivity on fiber size and myonuclear number in rat soleus muscle. J Appl Physiol. 2005;99:1494–9.

34. Psatha M, Wu Z, Gammie FM, Ratkevicius A, Wackerhage H, Lee JH, Redpath TW, Gilbert FJ, Ashcroft GP, Meakin JR, Aspden RM. A longitudinal MRI study of muscle atrophy during lower leg immobilization following ankle fracture. J Magn Reson Imaging. 2012;35:886–95.

35. Henriksson-Larsen KB, Lexell J, Sjostrom M. Distribution of different fibre types in human skeletal muscles. Method for the preparation and analysis of cross-sections of whole tibialis anterior. Histochem J. 1983;15:167–78.

36. De Jonghe B, Sharshar T, Lefaucheur JP, Authier FJ, Durand-Zaleski I, et al. Paresis acquired in the intensive care unit: a prospective multicenter study. JAMA. 2002;288:2859–67.

37. Petrasek PF, Homer-Vanniasinkam S, Walker PM. Determinants of ischemic injury to skeletal muscle. Method for the preparation and analysis of cross-sections of whole tibialis anterior. Histochem J. 1983;15:167–78.

38. Krawiec BJ, Frost RA, Vary TC, Jefferson LS, Lang CH. Hindlimb casting decreases muscle mass in part by proteasome-dependent proteolysis but independent of protein synthesis. Am J Physiol Endocrinol Metab. 2005;289:E969–80.

39. Parry SM, El-Ansary D, Cartwright MS, Sarwai A, Berney S, et al. Ultrasoundography in the intensive care setting can be used to detect changes in the quality and quantity of muscle and is related to muscle strength and function. J Crit Care. 2015;30:1151.e9–14.

40. Pillen S. Skeletal muscle ultrasound. Eur J Transl Myol. 2010;1(4):145–55.

41. Allingstrup MJ, Esmailzadeh N, Wilkens Krudsen A, Espensen K, Hartvig Jensen T, Wiss J, et al. Provision of protein and energy in relation to measured requirements in intensive care patients. Clin Nutr. 2012;31:462.e8.

42. Mansoor O, Breulde D, Bechereau F, Buffiere C, Pouyet C, Beaufriere B, et al. Effect of an enteral diet supplemented with a specific blend of amino acid on plasma and muscle protein synthesis in ICU patients. Clin Nutr. 2007;26:301–40.

43. Hoffer LJ, Bistrian BR. Appropriate protein provision in critical illness: a systematic and narrative review. Am J Clin Nutr. 2012;96:591.e600.

44. Nicolo M, Heyland DK, Chittams J, Sammarco T, Compher C. Clinical outcomes related to protein delivery in a critically ill population: a multicentre, multinational observational study. JPEN J Parenter Enteral Nutr. 2016;40:45–51.

45. Sreel SY, Beddoe AH, Hill GI. Aggressive nutritional support does not prevent protein losses despite fat gain in septic intensive care patients. J Trauma. 1987;27:262–6.

46. Hart DW, Wolf SE, Henndon DN, et al. Energy expenditure and caloric balance after burn. Increased feeding leads to fat rather than lean mass accretion. Ann Surg. 2002;235:152–61.

47. Casar MP, Langouche L, Coudyzer W, Vanbeekvoort D, De Dobelbaer B, Guiza FG, et al. Impact of early parenteral nutrition on muscle and adipose tissue compartments during critical illness. Crit Care Med. 2013;41:2298–309.

48. Dock W. The evil sequelae of complete bed rest. JAMA. 1944;125:1083–5.

49. Reid MB, Moynan JS. Beyond atrophy: redox mechanisms of muscle dysfunction in chronic inflammatory disease. J Physiol. 2011;589:2171–9.